Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

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Abbreviations

Abbreviation	*Description*
ACh	acetylcholine
ACTH	adrenocorticotropic hormone
AHA	american heart association
ANS	autonomic nervous system
CABG	coronary artery bypass graft
CAD	coronary artery disease
CMV	cytomegalovirus
CNS	central nervous system
CPn	*chlamydia pneumoniae*
CRH	corticotrophin-releasing hormone
CRP	C-reactive protein
CV	coefficient of variation
EBV	epstein barr virus
EDTA	ethylenediaminetetraacetic
ELISA	enzyme-linked immunosorbent assay
EXIT	exhaustion intervention trial
HF	high-frequency
HPA	hypothalamic-pituitary-adrenal
HRV	heart rate variability
HSV	herpes simplex virus
IgG	immunoglobulin G
IL	interleukin
LDL	low density lipoprotein
MI	myocardial infarction
MIF	macrophage migration inhibitory factor
MIVE	maastricht interview for vital exhaustion
MQ	maastricht questionnaire
PB	pathogen burden
PCI	percutaneous coronary intervention
RCT	randomized controlled trial
RR	relative risk
SMC	smooth muscle cell
TMB	tetramethylbenzidine-solution
TNF	tumor necrosis factor
VE	vital exhaustion
VNS	vagus nerve stimulation
VZV	varicella zoster virus

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General introduction, aims and outline of thesis

General introduction

Coronary artery disease (CAD) is the result of atherosclerosis. Atherosclerosis is a chronic inflammatory disease of the medium-sized and larger elastic and muscular arteries [1] central in the pathogenesis of CAD. Atherosclerosis starts in early life and spans over decades. Clinical events resulting from atherosclerosis, like stroke and cardiovascular diseases, in particular CAD, pose a serious health problem all over the world. Because these clinical events are so prevalent and have such an impact on the health care system much research into the causes of these clinical events is undertaken. In case of CAD, atherosclerosis may generate plaques that may obstruct the coronary arteries, and consequently decrease the blood-flow depriving the heart tissue from oxygen leading to damage to the heart muscle. Most damage is caused when the plaques become unstable and rupture. Main clinical manifestations of CAD are (unstable) angina pectoris, acute myocardial infarction and sudden cardiac death. A number of strategies to treat these clinical manifestations can be applied. Most patients undergo a percutaneous coronary intervention (PCI or angioplasty). PCI is a procedure to increase blood-flow in the arteries by inflating a balloon. In addition to inflation, most often the cardiologist places a stent to ensure the dilated area to remain open. A more invasive treatment is a coronary artery bypass graft (CABG or open heart bypass surgery), these mechanistic treatments are used in combination with drug therapy and ever more often with lifestyle modification training.

Atherosclerosis and the development of CAD

The development of atherosclerosis is called atherogenesis. It is long known that atherosclerosis is associated with inflammation [2, 3]. The response-to-injury theory by Ross [1] is the most commonly used theory that describes the development of atherosclerosis. It postulates that endothelial dysfunction is the first step in atherosclerosis, each characteristic lesion of atherosclerosis represents a different stage of the chronic inflammatory process in the artery. The second step is the formation of fatty streaks followed by the formation of advanced, complicated lesion and finally by the development of unstable fibrous plaques.

Endothelial dysfunction

In the normal, healthy arterial wall three layers exist. The outer layer is called the adventitia, merely composed of concentrically layered elastic fibers (other components are lymph vessels and nerves). The middle layer is called the media, it is mainly composed of smooth muscle cells (SMC), elastin and collagen. The inner layer is called the intima, bordering the media with basal lamina and the endothelium with the lumen. Blood flows through the lumen. Main task of the endothelium is maintaining the vascular architecture, to accomplish this task, the endothelium can respond to injury. The earliest changes that precede the formation of lesions in atherosclerosis take place in the endothelium [1]. Functional and structural changes (e.g. shear stress, adhesiveness of the endothelium) cause endothelial dysfunctioning. Endothelial dysfunctioning can lead to injury, including increased endothelial permeability to lipoproteins and other plasma constituents. Furthermore, the adhesion of leukocytes and migration of these leukocytes into the artery wall. Because of the injury the endothelium changes to procoagulant instead of anticoagulant properties and forms vasoactive molecules, cytokines, and growth factors.

Formation of fatty streaks

Local elevations of the intima occur when migrated monocytes differentiate into a specific subset of macrophages that express scavenger receptors and start the uptake of oxidized low-density lipoprotein (oxLDL). The uptake of oxLDL leads to foam cells and an accumulation of foam cells in combination with T lymphocytes forms the first visible manifestation of atherosclerosis, namely a fatty streak. The fatty streak is an inflammatory condition because the foam cells and the activated T lymphocytes produce a large number of pro-inflammatory mediators such as cytokines, growth factors, chemokines and proteolytic enzymes in the sub-endothelial layer. By expressing these mediators more leukocytes migrate from the lumen into the sub-endothelial layer and more platelets adhere to the dysfunctional endothelium.

Formation of an advanced, complicated lesion

If the inflammatory response does not effectively neutralize or remove the offending agents, it can continue indefinitely [1]. SMCs migrate from the media to the sub-endothelial layer, start growing and proliferate due to growth factor release from activated monocytes. SMCs become intermixed with the area of inflammation to form an intermediate lesion. SMCs start expressing matrix proteins that lead to the formation of a fibrous cap because endothelial cells overlying the SMCs gradually decline. The fibrous cap covers a mixture of leukocytes, lipid, and debris, which may form a necrotic core [1]. The arterial wall thickens, but initially the vessel lumen is not affected due to gradual dilation (i.e. compensatory enlargement), a process called “remodeling”. Eventually, the vessel lumen diminishes and blood flow to the heart tissue decreases, a critical stage in atherogenesis.

Development of unstable fibrous plaques

Activated macrophages in the plaque release pro-inflammatory cytokines, chemokines, growth factors, metalloproteinases and other proteolytic enzymes. Especially the metalloproteinases and the proteolytic enzymes cause degradation of the matrix leading to hemorrhage from the vasa vasorum or rupture of the fibrous plaque and can result in thrombus formation and occlusion of the artery. Rupture of the fibrous cap or ulceration of the fibrous plaque can rapidly lead to thrombosis (as a natural response to injury) and are complications in advanced lesions that lead to unstable coronary syndromes or myocardial infarction (MI) [4-7]. In conclusion, atherogenesis is a long-term process, in which a central role is attributed to infiltration, activation and proliferation of monocytes/macrophages. Although these cells are central in atherogenesis, different cell types, like T cells, SMCs endothelial cells and platelets are also crucial in atherogenesis. All of these cell types are capable of producing both pro- and anti-inflammatory mediators that can ultimately lead to a vulnerable plaque.

Inflammatory mediators in CAD

The understanding of atherosclerosis as an inflammatory disease led to a series of seroepidemiological studies. Inflammatory mediators were measured and tested for their prognostic value of future outcome. These risks help physicians with identifying patients at a higher risk of recurrent cardiac events, and therewith provide the physician with an extra tool to provide better health care to the patient. Seroepidemiological studies do, however, oversimplify very complex immunological pathways and cross-links between multiple pathways. Therefore, seroepidemiological studies must always be interpreted with caution. In CAD patients both pro- and anti-inflammatory mediators are increased in different stages of atherosclerosis, suggesting a pro-inflammatory state in these patients [8-10]. In the following paragraphs, a number of inflammatory mediators in CAD are reviewed.

TNF-α complex

TNF is an endogenous pro-inflammatory cytokine that stimulates the production of other endogenous cytokines, such as IL-1β and IL-6. Macrophages are the predominant source of TNF-α, but other cell types can produce the pro-inflammatory cytokine as well [11]. TNF-α is a protein of 185 amino acids glycosylated at positions 73 and 172. It is synthesized as a precursor protein of 212 amino acids. Monocytes express at least five different molecular forms of TNF-α with molecular masses of 21.5-28 kDa. They mainly differ by post-translational alterations such as glycosylation and phosphorylation.

Binding of TNF-α to TNF-α p55 and p75 receptors mediates the biologic effect of TNF-α. TNF gene expression is controlled by NF-κB, AP-1 and cJun via the TRAF-TRADD cascade [12]. Shedding the TNF-α receptors by metalloproteinases produces soluble p55 and p75 [13-15]. The loss of extra cellular domains leads to fewer functional receptors and diminished cell responsiveness to TNF-α [16, 17]. TNF-α in serum can bind to both soluble TNF-α receptors and form the TNF-α complex. The TNF-α complex prohibits TNF-α to exert its pro-inflammatory action, but can also prolong the TNF-α half-life [18]. This complex is not very stable [19] when compromised TNF-α can bind to the membrane bound TNF-α receptors.

Seroepidemiological studies have shown that a high concentration of TNF-α is a consistent risk factor of future cardiac events in various clinical conditions (e.g. in asymptomatic, otherwise healthy subjects; in patients with chronic heart failure) [20-22]. In addition to these studies, there are a number of studies that not only reported a prognostic value for TNF-α, but also for its’ soluble receptors [23-26]. These studies indicate that high concentrations of TNF-α in healthy subjects, but also in cardiac patients are damaging to the heart. This resulted in trials to neutralize TNF-α in cardiac patients. All trials these trials were stopped by authorities because of a lack of result or even negative results [27-32]. This example highlights why results of seroepidemiological studies must be treated with extreme caution and stresses that even scientists tend to oversimplify very complex immunological pathways.

IL-6 complex

Interleukin 6 (IL-6) is a cytokine that plays a central role in differentiation and growth of numerous cells like B- and T-cells, hematopoietic precursor cells and neuronal cells. Various cells including endothelial cells secrete IL-6 under the influence of other cytokines like TNF-α and IL-1β. Human IL-6 is a 26-kDa glycoprotein with two potential N-linked glycosylation sites.
In vivo and in vitro IL-6 has pro- and anti-inflammatory capabilities. Like IL-10, IL-6 can inhibit pro-inflammatory cytokine responses (such as LPS-induced IL-1β and TNF-α [33-35]) and enhance the release of anti-inflammatory molecules such as anti-protease inhibitors [36]. IL-6 is a potent inducer of acute phase proteins formed after acute inflammation, one of these, C-reactive protein (CRP) is mainly produced in the liver. The biologic effect of IL-6 is mediated through binding to soluble IL-6 receptor (sIL6r or gp80) forming the IL-6 complex. sIL6r is formed by shedding the IL-6 receptor from its' cells (e.g. hepatocytes, neutrophils and monocytes). The IL-6 complex protects IL-6 from enzyme inactivation. The IL-6 complex can bind to gp130. The binding induces a disulfide-linked homodimerization of gp130. gp130 is expressed on almost all cell types. Cells are activated through trans-signaling; the gp130 dimer is the signal transducing protein present on the cell membrane. The activated IL-6-sIL6r-gp130 receptor complex activates the JAK/STAT and MAPK signal transduction cascade, leading to DNA transcription.
Like for TNF-α, seroepidemiological studies have shown that high concentrations of IL-6 are a risk factor of CAD. These studies were performed in various clinical conditions (e.g. in asymptomatic, otherwise healthy subjects; in patients with peripheral and stable CAD and in patients with unstable angina) [20, 21, 23, 25, 26, 37-39]. A role for IL-6 in the pathogenesis of CAD is probably through a combination of autocrine, paracrine and endocrine mechanisms [40].

C-reactive protein

CRP is a pro-inflammatory acute phase protein. The term “acute phase” refers to local and systemic events that accompany inflammation. Local responses include neutrophil chemotaxes, release of lysosomal enzymes and vasodilatation. Systemic responses include an upregulation of acute phase proteins, fever and leukocytosis. CRP is a 105 kDa, five non-glycosylated subunit containing polypeptide, shaped in a disc-like manner. CRP is mainly produced by hepatocytes under the influence of cytokines, particularly IL-6 [41] and to a lesser extent IL-8 [42].
The biological role of CRP is not clear, but it is known to bind to numerous ligands. No CRP receptor was found so far. Most of the known ligands binding to CRP are modified, damaged or extrinsic, therefore CRP might contribute to the hosts' defense against infection. Although its' mechanism is not known, of all inflammatory markers, CRP is considered to be the most powerful predic-

tor of future cardiac events. CRP is the only inflammatory marker for which the American Heart Association (AHA) has issued clinical cut-off points. Categories are: low concentrations of CRP (<1 mg/L), average concentrations of CRP (1.0-3.0 mg/L) and high concentrations of CRP (>3.0 mg/L), subjects with high concentrations are at high risk of future cardiac events [43]. The AHA issued these guidelines based upon large seroepidemiological studies that showed a high prognostic value for high concentrations of CRP [20, 25, 44-52].

IL-1 receptor antagonist

Interleukin 1 receptor antagonist (IL-1ra) is an anti-inflammatory cytokine produced by monocytes and macrophages after stimulation by LPS, IL-4, IL-6, IL-10 and IL-14. IL-1ra is a 152 amino acid, 22-kDa glycosylated protein [53]. The IL-1RI receptor forms when bound to IL-1α or IL-1β a dimer with IL-1R receptor accessory protein (IL-1AcP); starting intracellular signaling. When IL-1ra binds to IL-1RI, no dimer is formed and therefore no intracellular signaling takes place. Due to the competitive binding to the IL-1RI receptor, IL-1α or IL-1β cannot bind [53]. After LPS stimulation IL-1ra is synthesized 100 fold less than IL-1α and IL-1β, but the IL-1α and IL-1β half-life is only 20 minutes whereas the half-life of IL-1ra is much longer [54]. Seroepidemiological studies by Patti et al established IL-1ra as a risk factor of CAD [55-58].

IL-10

Interleukin 10 (IL-10) is an anti-inflammatory cytokine produced by T helper cell type 2 (Th2) $CD4^+$ cells. IL-10 blocks the synthesis of macrophage-derived pro-inflammatory cytokines like IL-1β, TNF-α and IFN-γ by promoting degradation of messenger RNA for these pro inflammatory cytokines [59]. Synthesis of the chemokine IL-8 is also inhibited by IL-10. NF-κB is stopped from translocating to the nucleus by IL-10 [60, 61]. MHC II class expression and cell surface expression of co-stimulatory molecules on Th1 cells is diminished by IL-10 [62]. IL-10 is a 18,5-kDa-homodimeric protein with little or no N linked carbohydrate.

IL-10 exerts its action by binding to the IL-10 receptor, which has a 90 to 110 kDa molecular weight. After IL-10 receptor phosphorylation second messengers are activated. The JAK/STAT pathway mediates the IL-10 signal transduction much like IL-6. IL-10 deficient mice develop chronic enterocolitis and are growth retarded [63, 64]. Because of the anti-inflammatory actions of IL-10, it is considered to have a protective role in atherosclerosis. The few seroepidemiological studies investigating the prognostic role of IL-10 have confirmed the protective role of IL-10 [65-67]. High concentrations of IL-10 in these studies were cardio protective.

IL-8

Interleukin 8 (IL-8) is a pro-inflammatory chemokine produced by macrophages, lymphocytes, endothelial cells and neutrophils, induced by TNF-α and IL-1β. IL-8 plays a central role in inflammation by attracting monocytes, neutrophils, endothelial cells, T-cells and mediates acute-phase protein produc-

tion [42]. IL-8 is a member of the chemokine ligand family. Chemokines are chemotactic cytokines involved in leukocyte trafficking and activation. IL-8 is a small 8 kDa, 72 amino acid chemokine.
IL-8 exerts its action by binding to the CXCR1 and CXCR2 receptors on the targeted cell, leading to up regulation of high affinity integrin at the cell surface of the targeted cell [68]. The targeted cell adheres to the epithelial layer. MCP-1 another chemokine binds to CCR2 at the surface of the targeted cell, leading to migratory behavior. The chemokine super family is responsible for neutrophil adhesion, transmigration through endothelium, chemotaxis, respiratory burst and lysosomal degranulation. IL-8 mediates the transport of T-cells into the inflammatory region [69]. Qi et al [70, 71] and Dominguez-Rodriguez et al [72] found a prognostic value for IL-8 in association with future cardiac events.

Neopterin

Neopterin is a member of the pteridine family. Pteridines are small chemical compounds ubiquitously distributed and can act as inhibitors of xanthine oxidase [73], and therewith reduce free radical formation. The biological role of neopterin is not clear, but it can induce iNOS expression and thus promote the number of free radicals [74, 75] by translocating NF-κB [76]. Neopterin can therefore induce and inhibit indirect damage by free radicals and formation of pro-inflammatory cytokines.
Neopterin is produced in IFN-γ stimulated macrophages [77] and often used as an inflammatory marker. Neopterin does not undergo extravasation and is released to body fluids and excreted via the kidneys, therefore it is a good marker for immune-mediated inflammation [78]. Seroepidemiological studies found a role for neopterin in CAD. In humans there is a positive association between serum neopterin concentration and the extent of carotid atherosclerosis [79]. The levels of neopterin are elevated in patients with acute as well as chronic CAD [80-82]. In stable CAD, only small amounts of neopterin are produced due to the small number of active macrophages [83]. Gurfinkel et al found a positive association between neopterin levels and the number of affected coronary arteries. Elevated baseline level of serum neopterin is a better predictor of a non-Q-wave MI than the initial electrocardiogram [84].

Role of pathogens in atherosclerosis

Evidence that pathogens play a role in atherosclerosis emerged in the late seventies when Fabricant et al. observed development of atherosclerotic lesions following Marek’s disease infection in chickens [85]. Over the last two decades numerous studies were conducted linking individual pathogens to atherosclerosis. Infectious agents that have been implicated in the pathogenesis of CAD include the bacterium *Chlamydia Pneumoniae* (CPn) and herpes viruses such as Herpes Simplex virus (HSV), Varicella Zoster virus (VZV), Epstein-Barr virus (EBV), and Cytomegalovirus (CMV) [86]. Characterized by lifelong infections, these pathogens can exert direct effects on atherogenesis by residing in the vascular wall, most likely after being delivered to the vessel wall by circulating monocytes. These effects include increased proliferation and migration of smooth muscle cells (SMC), inhibition of apoptosis, dysfunction of endothelial cells with pro-coagulant effects, increased cholesterol loading in macrophages and SMC, contribution to plaque rupture, and increased expression of chemokines, adhesion receptors, reactive oxygen species and pro-inflammatory cytokines [87]. In addition, these pathogens may indirectly contribute to CAD by fostering systemic inflammation, which may damage vascular walls (e.g., by cytokines and proteases) and lead to a procoagulant state [88], by immune-mediated mechanisms (e.g., molecular mimicry) [89], and by altering serum lipids toward a more proatherogenic profile [90]. Notwithstanding this evidence, an increased risk of CAD has not consistently been found for each of these individual pathogens [91-93]. Because atherosclerotic plaques often harbour multiple pathogens, including HSV, CMV, and CPn [94, 95], recent seroepidemiological studies have focused on CAD risk and the aggregate number of atherogenic pathogens to which individuals have been exposed during their life (i.e. the pathogen burden; PB) [96-100]. Although negative results have been reported [101, 102], most of these studies showed that an increased PB raises the risk of CAD. This raises the question why PB increases this risk because this marker does not show whether viral and/or bacterial infections are active, but only that individuals have been exposed to a number of pathogens during their life. One suggestion came from a study by Zhu et al., who showed that CAD prevalence is increased only in those patients who have both evidence of prior pathogen infection (seropositivity) and elevated CRP levels [103]. This was also found by Prasad et al, who combined high concentrations of CRP with PB and showed that patients with high PB and high CRP had higher odds of future CAD [104] and other studies in which the coupling of pathogens with CRP confer a strong risk of morbidity and mortality [89, 91]. These findings, therefore, suggest that these pathogens may play a causal role in the genesis of atherosclerosis [103] through the inflammatory response they provoke.

Psychological risk factors in CAD

In addition to inflammatory markers and multiple pathogens as risk factors, more traditional risk factors (e.g. smoking, hypertension, hyperlipidaemia, family history of CAD, diabetes and a sedentary lifestyle) are important for the development of atherosclerosis. The absence of these factors in over 50% of patients with atherosclerosis indicates that additional risk factors remain to be identified for CAD [87, 105, 106]. In the last decades psychological risk factors were added to the list of risk factors of CAD. The psychological risk factors include depression, vital exhaustion, anxiety, hostility, social isolation and work strain [105, 107, 108]. Because the sub-studies reported in this thesis are focused on vital exhaustion, the following paragraphs will discuss this psychological risk factor and the results of the EXhaustion Intervention Trial (EXIT), a randomized controlled trial designed to test the hypothesis that intervening on exhaustion reduces the risk of a new coronary event. In addition, the overlap between vital exhaustion and clinical depression is discussed and psychobiological pathways that may underlie the association between exhaustion and future CAD are reviewed.

Vital Exhaustion

A fatal or non fatal MI only rarely strikes out of the blue and is most often preceded by chest pain and feelings of unusual fatigue, irritability and general malaise. In a cardiac population the prevalence rate of patients that experience undue fatigue or a lack of energy before a cardiac event range from 36-50% [109]. Clinical interviews with MI patients about the mental precursors of MI led to the formulation of the concept of vital exhaustion, a concept that summarizes the unusual fatigue, irritability and general malaise [110]. To measure exhaustion, a 37 item standardized self-administered instrument, the Maastricht Questionnaire (MQ), has been developed [111]. Table 1 presents the ten most prevalent complaints as observed with the MQ in 3584 angioplasty patients two weeks after PCI. Most of these complaints are related to exhaustion. These data were collected in the EXIT study [112].

Table 1. Top 10 mental complaints after PCI in screened exhausted patients

Complaint	*Prevalence*
Often feel tired	63%
I don't have what it takes	59%
Listlessness	50%
Feel weak all over	49%
Troubles staying asleep	49%
Not accomplishing much	48%
Minor hassles irritate easily	47%
Body feels like a battery that is losing its power	47%
Don't feel fine	46%
Can't cope with everyday problems	44%

Many researchers approach the complaints as portrayed in Table 1 as a clinical depression. The prevalence of major depression in patients with CAD is approximately 20% [113-116], whereas the complaints in Table 1 are present in 36-50% of CAD patients [109]. Elevated depressive symptoms appear to be a robust risk factor of CAD [117-120]. The increased risk not only stands for depressed cardiac patients [121-127], but also in depressed, otherwise healthy subjects [128-132]. Although there are strong epidemiological arguments to approach the feelings of CAD patients as a clinical depression there are also arguments to avoid an interpretation of these feelings as a mental disorder. In a study by van Diest et al. the Profile of Mood States was used to investigate whether a depressed mood was present in exhausted subjects, the depressed mood was almost absent in exhausted subjects [133]. Only 20% of exhausted subjects met the DSM criteria for major depression. However, nearly all subjects who feel depressed also meet the criteria for vital exhaustion [107]. Table 2 shows the association between depression and vital exhaustion as observed in the EXIT study. All depressed patients felt exhausted. However, of those who were exhausted only 11% was depressed. From a clinical point of view it should be noted that cognitive distortions that are characteristic of a melancholic depression, such as: "I do not deserve to be loved" or "I am a failure" are rare in CAD patients.

Table 2. Association of Exhaustion with depression at 18 months

	Exhausted	*Not exhausted*
Depressed	33	0
Not despressed	268	333

$\chi^2 = 38.52$ $p < 0.01$

Furthermore, the immunological characteristics of CAD patients do not fit well into the neurohormonal characteristics of melancholic depression. CAD is an inflammatory disease. Therefore it is hard to conceive that a mental state that is associated with anti-inflammatory properties is a risk factor of CAD. Available evidence suggests that (near future) CAD patients are characterized by decreased activity of the HPA axis, a state that activates immune-mediated inflammation (see below). The concept of vital exhaustion corresponds well with atypical depression, a form of depression that is not or to a much lesser degree characterized by mood disorders. For these reasons it is argued that vital exhaustion is conceptually different from depression.

Epidemiological studies of vital exhaustion as risk factor of CAD

Over the years vital exhaustion has proven to have significant prognostic value for cardiovascular disease endpoints. Vital exhaustion has been found to be predictive of nonfatal MI and cardiac mortality in apparently healthy subjects, and to increase the risk of recurrent cardiac events in MI patients and in patients undergoing PCI [134-138]. In a secondary analysis of the MQ in the first study that showed a prognostic value for vital exhaustion [139], the fatigue component of the MQ was the component with the highest prognostic value [140]. In fact, the depression and irritability subscales lost their prognostic value when controlling for fatigue. Among the epidemiological studies that tested the hypothesis that VE increases the risk of CAD an important place is occupied by EXIT. Because all data to be reported in this thesis were collected in EXIT, a more detailed description of that study is presented below.

In the EXIT study 710 exhausted PCI patients in four different centers were included and randomized over a “care as usual” and a “psychological intervention” group (n=344 vs. n=366) [112]. The aim of the intervention was to reduce stressors that cause exhaustion and to support recovery by promoting rest and by making rest more efficient. Group discussions were used to identify stressors in the family and work domain, and to help the patient in coping with these stressors. Recovery was supported by discussions about the minimum and maximum length of resting time, by relaxation exercises designed to make rest more efficient [141], by stimulating physical exercise, and by home work assignments. Hostility, a major cause of exhaustion, was treated by methods developed by Powell and by Williams and Williams [142, 143]. Each group consisted of six patients. They met weekly during 10 weeks and monthly for the following 4 months. The partners were encouraged to participate in the sessions.

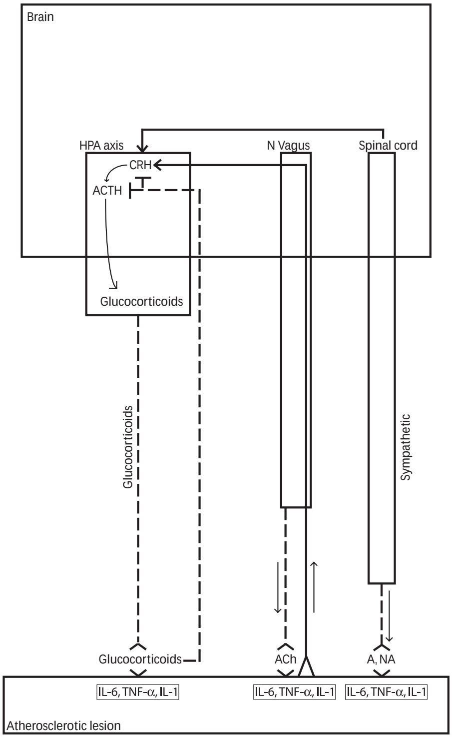
EXIT demonstrated that patients who improved on feelings of exhaustion between baseline and 6 months had lower risk of a recurrent cardiac event. This effect was not mediated by the behavioral intervention. The intervention reduced the odds of remaining exhausted at 18 months by 56% in those without a previous history of CAD (OR=0.44; 95%CI .29-.66; $p<0.00$), but had no effect on exhaustion in those with a history of CAD (OR=0.93; 95%CI .56-1.55; p=0.78). The intervention did not reduce the risk of a new coronary event within 2 years (RR=1.14; 95%CI .82-1.57; p=0.44). Post-hoc analyses suggest that the effect of the intervention was limited by a positive history of CAD, the presence of a chronic, painful condition (especially rheumatism), and by opposite effects on early and late cardiac events. The result of this post hoc analysis showed that the intervention reduced the risk of a late event by 55% (RR=0.45; 95%CI: .19-1.06; p=0.07) in patients without a history of CAD or presence of a chronic, painful condition.

Putative psychobiological pathways between the exhaustion, immune system and CAD

Exhausted subjects are characterized by increased blood coagulability and decreased early morning fibrinolysis [144-146]. Concentrations of IL-1β, TNF-α, IL-6, and CRP are also increased in exhausted subjects [147-149]. In addition, lower concentrations of ACTH and cortisol, and reduced glucocorticoid sensitivity have been associated with exhaustion [147, 150-153], thus suggesting that exhausted subjects are characterized by a decreased activity of the HPA axis. Hypoactivity of the HPA axis activates immune-mediated inflammation [154, 155]. Therefore, a pro-inflammatory state may be a pathophysiological mechanism that underlies the increased risk of CAD in exhausted subjects as observed in epidemiological studies [135, 136, 138]. Finally, exhaustion has been linked to reactivation of microorganisms suspected of playing a role in the progression of atherosclerosis (Cytomegalovirus and *Chlamydia pneumoniae*) [148, 149]. In the next paragraph some insights into the possible association of HPA axis activity and CAD are presented.
The HPA axis and the sympathic-adrenomedular system are the two main arms of the stress system, which main function is to maintain basal and stress-related homeostasis. In response to stress, the sympathic-adrenomedular system is activated, which results in the release of adrenalin and noradrenalin. At the same time, corticotrophin-releasing hormone (CRH) is released by the hypothalamus (i.e. paraventricular nucleus), these two systems innervate and stimulate each other. After CRH release, it induces the anterior pituitary to excrete adrenocorticotropic hormone (ACTH). ACTH in turn stimulates the adrenal cortex to release glucocorticoids into the blood stream. Of the glucocorticoids, cortisol is physiologically the most relevant. The glucocorticoids inhibit synthesis, release and activity of IL-6, TNF-α, IL-1β and other pro-inflammatory mediators [154]. Glucocorticoids also play a role in atherogenesis: cell adhesion, migration, macrophage activation, antigen activation, T cell expression and activation, proliferation and differentiation, mature cell function and antibody production are all suppressed by glucocorticoids [156-158]. Glucocorticoids help in maintaining homeostasis by negative feedback loops, glucocorticoids inhibit the expression of CRH and ACTH and therewith the release of the glucocorticoids. Glucocorticoids are able to down regulate HPA axis activity.
HPA axis activity is characterized by a circadian rhythm, every hour two or three pulses of CRH are released. Early in the morning, the pulses are at their peak and increase the magnitude of ACTH and cortisol pulses. The pulses are lowest during late evening, but increase once again during the second half of nocturnal sleep. HPA axis output is also seasonal, mechanisms underlying the seasonal changes remain to be elucidated [159].

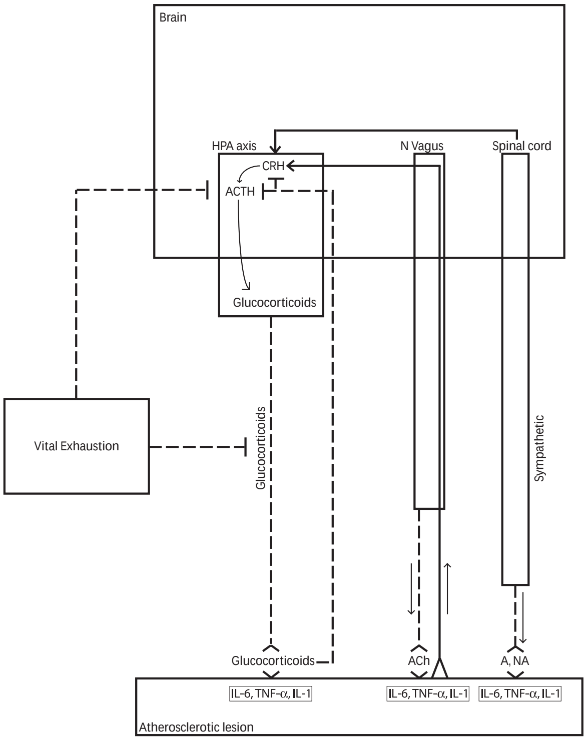
During acute stress (e.g. microbial invasion or injury), a bidirectional communication between the immune system and the nervous system through the nervus vagus causes sudden changes in the circadian rhythm. Tracey called this the "inflammatory reflex" [160]. The first reaction of the innate immune system to acute stress is to express pro-inflammatory cytokines by activated macrophages (immune-mediated inflammation). These pro-inflammatory mediators, mainly TNF-α, IL-1β and IL-6 can activate sensory fibers that ascend in the nervus vagus. If a certain threshold is reached, systemic humoral anti-inflammatory responses through activation of the HPA axis can be established. The release of glucocorticoids results in strong anti-inflammatory reactions to control the inflammatory response of the innate immune system [160]. Increased efferent signals in the nervus vagus suppress peripheral cytokine release through

Figure 1. Interactions between brain and an atherosclerotic lesion



This schematic figure shows the interactions between the atherosclerotic lesion and the brain. Dashed lines represent inhibitory pathways and black lines represent excitatory pathways. The atherosclerotic lesion is a chronic inflammatory site and is via 3 pathways connected to the brain. Firstly, the sympathetic nervous system is connected via the spinal cord using adrenalin (A) and noradrenalin (NA) as neurotransmitters, the sympathetic pathway stimulates the HPA axis to produce CRH and inhibits pro-inflammatory mediators at the site of the lesion. Secondly, during acute stress acetylcholine (ACh) is released by the nervus vagus and inhibits pro-inflammatory mediators at the lesion. The pro-inflammatory mediators can also stimulate afferent fibers in the nervus vagus and stimulate CRH production in the HPA axis. Thirdly, the HPA axis is stimulated to produce CRH, ACTH and glucocorticoids, which inhibit the pro-inflammatory mediators at the lesion, a negative feedback mechanism of the glucocorticoids controls the expression of glucocorticoids.

Figure 2. Interactions between brain and an atherosclerotic lesion in patients with vital exhaustion



As in figure 1, black lines represent excitatory pathways and dashed lines represent inhibitory pathways. In exhausted patients the HPA axis is hypoactive, which results in lower levels of glucocorticoids. Because of the low levels of glucocorticoids, IL-6, TNF-α and IL-1 are less inhibited and can possibly accelerate the progress of atherosclerosis. The biological mechanism behind the hypoactive HPA axis by vital exhaustion is not known.

macrophage nicotinic receptors and cholinergic anti-inflammatory pathway (acetylcholine). The efferent pathway of acetylcholine reacts instantly, whereas the afferent pathway of glucocorticoid production via the HPA axis takes hours to complete. To illustrate the interactions between the brain and CAD (depicted by an atherosclerotic lesion), Figure 1 was created. Figure 2 shows the interactions between the brain and an atherosclerotic lesion in patients with vital exhaustion.

Table 3 shows disturbances in the interaction between the HPA axis and the immune-mediated inflammation. An excessive HPA response to inflammation can mimic the state of stress or hypercortisolemia and thus increase susceptibility to infectious agents and tumors but enhance resistance to autoimmune or inflammatory disease. Conversely, a defective HPA axis response can mimic the glucocorticoid-deficient state and thus cause resistance to infections and neoplasms but increased susceptibility to autoimmune or inflammatory disease [154].

Table 3. States potentially associated with suppression or activation of immune-mediated inflammation through defects in the Hypothalamic-Pituitary-Adrenal (HPA) axis or its target tissues

Suppression of immune-mediated inflammatory reaction	***Activation of immune-mediated inflammatory reaction***
Increased HPA axis activity	*Decreased HPA axis activity*
Cushing's syndrome	Adrenal insufficiency
Melancholic depression	Rheumatoid Arthritis
Chronic alcoholism	Atypical or seasonal depression
Chronic stress	Vital exhaustion
Long-term excessive exercise	Chronic fatigue or fibromyalgia
Pregnancy (last trimester)	Post-traumatic stress disorder

Adapted from Chrousos, 1995 [130].

Aims of this thesis

CAD is the result of severe atherosclerosis, an inflammatory disease. The general question is whether the depressive symptomatology that precedes CAD in more than half of all cases, approached in this thesis as a state of exhaustion, fosters inflammation.

This question is approached in several ways. Firstly, we investigated whether a behavioral intervention aimed at the reduction of exhaustion has a beneficial effect on inflammation. Secondly, we investigated whether the possible beneficial effect of the behavioral intervention was mediated by increased nervus vagus activity as reflected by high frequency heart rate variability. Thirdly, we investigated whether a state of exhaustion is associated with macrophage migration inhibitory factor (MIF), MIF is a cytokine produced by cells of the immune system, but also produced by the anterior pituitary in a hormone like fashion. Fourthly, we investigated the association between exhaustion and pathogen burden because pathogens are suspected to play a role in the initiation and duration of inflammation. Finally, (if the data allowed) we also investigated the risk of a new coronary event associated with each of these factors.

Chapter overview

In chapter 2, the hypothesis whether elevated concentrations of IL-6, CRP, TNF-α, IL-1ra, IL-8, IL-10 and neopterin approximately one month after PCI increase the risk of late cardiac events in exhausted patients is tested. Results are compared to earlier studies in both healthy and cardiac subjects.

In chapter 3, the first behavioral intervention of its kind looking for the effect of the behavioral intervention on inflammation is introduced. The objective of the study is to test whether a behavioral intervention aimed at the reduction of exhaustion in patients with CAD reduces inflammation.

Heart rate variability (HRV), the rhythmic oscillations in heart rate, is validated as a non-invasive measure of cardiac autonomic modulation. The high frequency HRV is a reflection of nervus vagus activity. In chapter 4, the objective is to test, whether a possible beneficial effect of the behavioral intervention as introduced in chapter 3, is associated with increases in nervus vagus activity as reflected by high frequency HRV.

The HPA axis plays an important role in the interaction between the brain and the immune system. In chapter 5, macrophage migration inhibitory factor (MIF), both a product of the immune system and the HPA axis is related to exhaustion, other inflammatory markers and CAD. The objective of the study is to explore the direction of the association of MIF with CAD and of the direction of the association of MIF with a state of exhaustion, using blood samples of PCI patients.

Pathogens also play an important role in the development of atherosclerosis, but the precise role of these mediators is not known. The concept of pathogen burden reflects the number of pathogens to which an individual has been exposed during life. In chapter 6, pathogen burden in combination with inflammatory markers is studied. The objective of the study is twofold: the first object was tot test whether a combined index of PB and systemic inflammation (represented by CRP) increases the risk of late cardiac events, more than PB and CRP by itself. The second objective was to test whether an index of PB and infection-induced inflammation (represented by neopterin) increases the risk of late cardiac events, more than PB and neopterin by itself. In the last chapter, the results of the studies are critically reviewed and put into perspective including directions for future studies.

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Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

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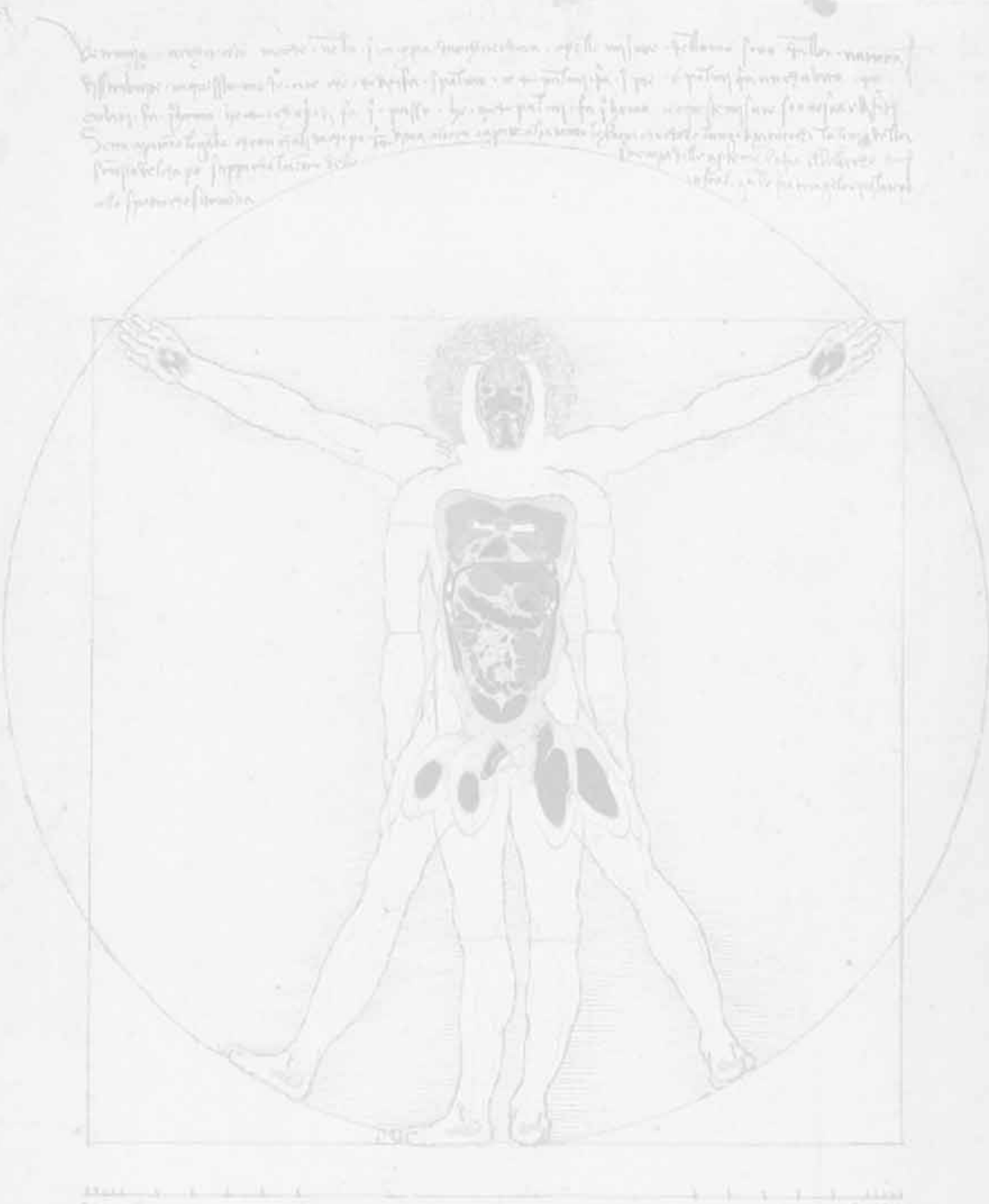

Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

Martijn Kwaijtaal

2

Inflammatory markers predict late cardiac events in patients who are exhausted after percutaneous coronary intervention

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Abstract

Chronic inflammation is one of the main underlying mechanisms in the development of coronary artery disease (CAD). We investigated the prognostic value of inflammatory markers for cardiac events occurring more than 6 months after percutaneous coronary intervention (PCI) (i.e. late cardiac events), furthermore we investigated the temporal stability of these markers. Exhausted patients (234) recently treated by successful PCI were studied. Serum samples collected about 6 weeks after PCI (baseline), 6 and 18 months after baseline were analyzed for CRP, IL-6, TNF-α, IL-10, IL-1ra, IL-8 and neopterin. In the mean cardiac follow-up of 24 months, 25 late cardiac events occurred. Cox proportional hazards analysis was used to determine the prognostic value. Elevated concentrations of IL-6 at baseline and 6 months later increased the risk of late cardiac events (RR 3.9, 95%CI 1.7-9.0, $p<0.00$ and RR 3.6, 95%CI 1.6-8.5, $p<0.00$). Elevated concentrations of CRP and IL-10 at baseline also increased the risk of late cardiac events (RR 2.5, 95%CI 1.1-5.7, $p=0.04$ and RR 2.5, 95%CI 1.1-5.6, $p=0.03$) as did IL-1 receptor antagonist at 6 months (RR 2.6, 95%CI 1.1-6.1, $p=0.04$). Temporal stability was high for most markers, but highest for IL-6. These results support the assumption that chronic inflammation is a pathophysiological mechanism in the development of CAD.

Key words: *angioplasty, restenosis, inflammation*

Introduction

Undue fatigue, increased irritability and feelings of general malaise reflect a state of exhaustion that precedes coronary artery disease (CAD) in 36-50% of all cases [1]. Exhaustion is an independent risk factor of non-fatal myocardial infarction (MI) in asymptomatic, otherwise healthy subjects, and of new cardiac events in patients undergoing percutaneous coronary intervention (PCI) [2-4].

A chronic pro-inflammatory status may underlie this association as concentrations of interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), IL-6, and C-reactive protein (CRP) are increased in exhausted subjects [5-7]. A pro-inflammatory status (as reflected by high concentrations of IL-6, CRP, TNF-α, IL-1 receptor antagonist (IL-1ra), IL-8 and neopterin) also raises the risk of future cardiac events in various clinical conditions (e.g. in asymptomatic, otherwise healthy subjects; in patients with peripheral and stable CAD and in patients with unstable angina)[8-13]. In these studies, maximum follow-up periods were up to 6 years in healthy subjects, and up to 6 months in most studies with PCI patients.

In 10 to 40% of PCI patients new cardiac events occur within 6 months (early cardiac events). These early events can be classified as an inadequate intervention, recoil of the vessel wall or neointimal hyperplasia (an effect of the vascular damage due to PCI) [14]. Transient increases in inflammatory markers (e.g. IL-6 and IL-8) occur within 48 hours after the PCI and raise the risk of early cardiac events. These post-procedural increases are due to the PCI itself and return to pre-procedural concentrations within a week [15]. Furthermore, post-procedural concentrations remain at pre-procedural concentrations up to at least one month after PCI [16], most likely because PCI does not remove the lesion.

Cardiac events occurring after 6 months (late cardiac events) are less frequently related to the culprit lesion, but to new lesions elsewhere in the coronary system, and reflect the progression of atherosclerosis [14]. Late cardiac events are therefore pathophysiologically different from early cardiac events and not caused by the PCI itself. As PCI does not remove the lesion and transient increases level off, we hypothesized that elevated concentrations of inflammatory markers one month after PCI have prognostic value for the occurrence of late cardiac events. As mentioned above, inflammatory markers are increased in PCI patients, however, little is known about the temporal stability of serum concentrations of these markers over an extended period after PCI. Therefore, we explored the temporal stability of these markers over a period of approximately 19 months after PCI.

The first objective of the present study was to test whether elevated concentrations of IL-6, CRP, TNF-α, IL-1ra, IL-8, IL-10 and neopterin approximately one month after PCI increase the risk of late cardiac events in exhausted patients. The second objective was to explore the temporal stability of these markers of inflammation after PCI.

Methods

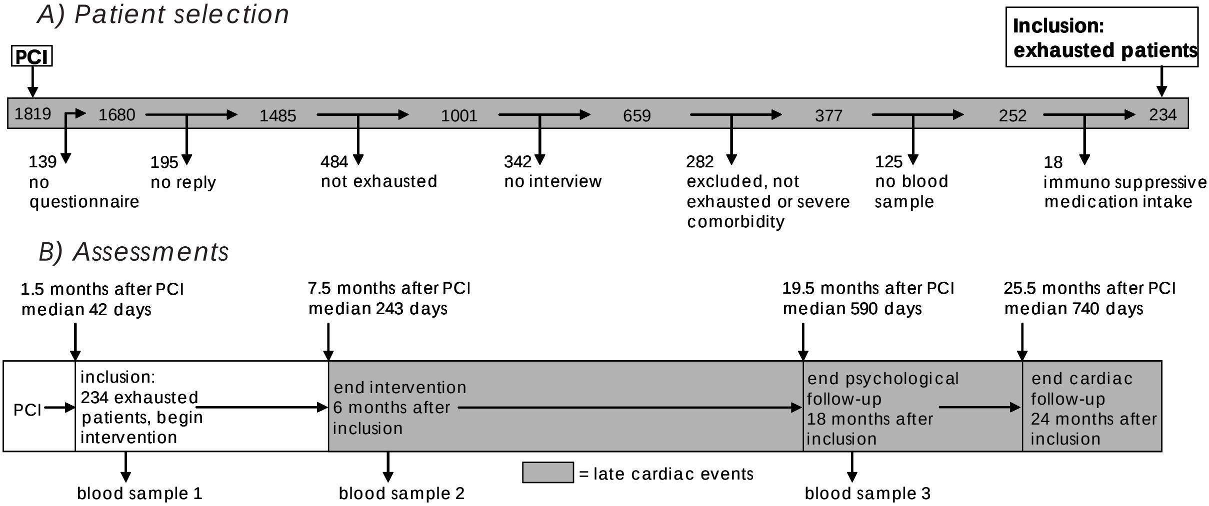
Participants

The present study is part of the Exhaustion Intervention Trial (EXIT), a multi-center randomized clinical trial [17]. Aim of EXIT was to test the hypothesis that reduction of exhaustion by a behavioral intervention reduces the risk of a new cardiac event in PCI patients. Participants of EXIT were 710 patients, aged 35-68 years, who felt exhausted after successful angioplasty (i.e. a reduction of 50% or more of the culprit lesion), without early complications. Exhaustion was measured in two phases. First, two weeks after PCI, patients were asked to complete the Maastricht Questionnaire (MQ), a standardized self-administered instrument designed to measure exhaustion [18]. Patients with a MQ score of 14 and higher were invited to participate in the second phase, which consisted of the Maastricht Interview for Vital Exhaustion (MIVE). MIVE has a higher predictive validity for future cardiac events than the MQ [19]. The inclusion criterion for EXIT was a MIVE score of at least 7. Exclusion criteria were: 1) severe somatic or mental comorbidity (e.g. renal insufficiency, a history of major depression of at least 3 years), 2) somatization disorder, fibromyalgia or chronic fatigue 3) participation in a behavioral rehabilitation program other than EXIT, 4) unsuccessful treatment for a recent mood or anxiety disorder, and 5) inability to speak Dutch. The mean interval between PCI and inclusion in EXIT was 31 days (median 27 days).

The intervention consisted of 10 weekly sessions with six patients, followed by four monthly sessions. The aim of the intervention was to reduce stressors leading to exhaustion, and to support recovery by promoting rest and by making rest more efficient. Group discussions, breathing-relaxation therapy, hostility therapy and educational sessions were applied as behavioral modification techniques.

For the present prospective sub-study blood samples were taken from 252 out of 377 patients in one of the participating centers (Maastricht). No selection bias was introduced because blood was collected of the last 252 consecutive patients. Of these 252 patients, 18 were excluded post-hoc because they were using immunosuppressive medication (i.e. medication exclusively developed for the purpose of suppressing the immune system). This meant that 234 patients were included in the prospective sub-study. The first blood collection (baseline) was approximately two weeks after inclusion (i.e. ± 46 days after PCI, median 42 days). The second blood collection was 6 months after baseline, 8 patients were missed due to loss-to-follow-up (e.g. patients did not show up, mistakes in lab while processing blood samples, death, patients refused to give blood), blood was collected of 226 patients. Last blood collection was 18 months after baseline, 55 patients were missed due to loss-to-follow-up, blood was collected of 179 patients (Figure 1). The institutional review board of the participating center approved the study protocol, and all participants gave written informed consent.

Figure 1. Flow chart of patient selection and assessments



Data collection and events

PCI treatments were conducted between 1996 and 2000. Follow-up of patients was completed in 2002, mean cardiac follow-up was 24 months. Occurrence of new cardiac events (defined as re-PCI, coronary artery bypass graft surgery (CABG), myocardial infarction (MI) or cardiac death) was assessed through inspection of the medical records by a research assistant in cooperation with a cardiologist (third author). To ensure that no deaths were missed, the family physicians received a letter asking whether a patient was still alive and, if the patient had died, what had been the cause of death. A 100% cardiac follow-up and cause of death was achieved, so no loss-to-follow-up or loss of events occurred. Late cardiac events were defined as new cardiac events occurring 6 months after inclusion in the study. Cardiac events occurring within 6 months after inclusion were excluded from the present analyses, because a number of events occurring within 6 weeks after PCI were missed due to the design of the study.

Immunological Techniques

Blood was allowed to clot at room temperature and centrifuged. Serum samples were stored at -20°C in anticipation of further processing. The markers of inflammation were measured in serum using quantitative enzyme-linked immunosorbent assay (ELISA). ELISA-kits were purchased from DiaMed Eurogen (Turnhout, Belgium). Serum for CRP was diluted (1000x) whereas the remaining inflammatory markers were quantified in undiluted serum. Test samples and standard solutions were incubated on pre-coated ELISA plates. A biotinylated secondary antibody against the relevant inflammatory marker was used. Peroxydase conjugated streptavidin was then applied to bind to the biotinylated antibody and after washing, a tetramethylbenzidine-solution (TMB) was used to stain the remainder of the bound streptavidin; the reaction was stopped by adding sulfuric acid. Optical densities were read using a PowerWaveX Reader (MWG Biotech, Ebersberg, Germany), data were calculated using KC4 software (MWG Biotech, Ebersberg, Germany).

Neopterin was measured using a competitive ELISA (IBL-Hamburg, Hamburg, Germany) in accordance with the manufacturer's recommendations. All serum samples were analyzed in duplicate. The sensitivity was calculated for all tests and values below sensitivity were raised to sensitivity value. The intra assay variation for all tests was less than 10%.

Statistical Analysis

To explore demographic or medical characteristics that may confound the association between inflammatory markers and the occurrence of late cardiac events, ANOVA and χ^2 were used to compare groups with and without cardiac events. Concentrations of inflammatory markers were not normally distributed, and cut-off values have not been reported for most of them. Therefore values were categorized in quartiles [11, 12]. For CRP, a cut-off value of 3.0 mg/l was applied as reported previously [20]. Top quartile groups were coded as 1 and the lower three quartiles were coded as 0. For CRP, concentrations above and below the cut-off were coded as 1 and 0 respectively. To assess the relative risk (RR) of late cardiac events associated with the inflammatory markers, Cox proportional hazards analyses were used to analyse time elapsed before the cardiac event.

Table 1. Demographic and medical characteristics at baseline of exhausted PCI patients with late cardiac events and exhausted PCI patients without cardiac events

	Late event n=25	No event n=188	p
Demographics			
Age	53.6 (8.0)	53.6 (7.3)	0.99
BMI	27.7 (4.3)	27.0 (4.0)	0.37
Blood pressure			
Systolic	137.6 (18.9)	130.5 (18.7)	0.08
Diastolic	88.6 (12.4)	82.2 (10.3)	0.01
Gender			
Male	18 (72%)	149 (79%)	0.41
Female	7 (28%)	39 (21%)	
Smoking			
Current	6 (24%)	36 (19%)	0.82
Stopped	17 (68%)	133 (71%)	
Never	2 (8%)	19 (10%)	
Medical characteristics			
Diabetes	1 (4%)	21 (11%)	0.27
Major depression	6 (24%)	28 (15%)	0.24
Chronic painful condition	5 (20%)	17 (9%)	0.10
Cardiac history			
Previous MI	7 (28%)	50 (27%)	0,22
Previous CABG	5 (20%)	13 (7%)	0.06
Previous PCI	5 (20%)	23 (12%)	0.28
Indication for PCI			
Stable Angina	5 (20%)	16 (9%)	0.45
Unstable Angina	13 (52%)	110 (58%)	
Myocardial Infarction	5 (20%)	39 (21%)	
Post MI angina	2 (8%)	23 (12%)	
Stenoses after PCI			
0	11 (44%)	88 (47%)	0.28
1	4 (16%)	55 (29%)	
2 or more	10 (40%)	45 (24%)	
Stent implanted	19 (76%)	117 (62%)	0.18
Medication 1.5 month (median 42 days) after PCI			
Ace inhibitor	5 (20%)	38 (20%)	0.98
Diuretics	5 (20%)	17 (9%)	0.10
Beta-blocker	18 (72%)	145 (77%)	0.57
Calcium antagonists	12 (48%)	67 (36%)	0.23
Statins	18 (72%)	143 (76%)	0.66
Nitrates	22 (88%)	147 (78%)	0.26
Aspirin	25 (100%)	179 (95%)	0.26
Acenocoumarol	0 (0%)	9 (5%)	0.00
Clopidogrel	1 (4%)	7 (4%)	0.98
Ticlopidine	4 (16%)	10 (5%)	0.04

Separate analyses were performed for the assessment at baseline and at 6 months. RRs and 95% confidence intervals (CIs) were adjusted for age, gender, stent placement, diabetes, BMI, current smoking and blood pressure. To explore the temporal stability of an inflammatory marker after PCI, non-parametric bivariate correlation (Spearman rank correlation coefficient) was calculated between baseline and 6 months and baseline and 18 months. Data processing and statistical analysis was performed using SPSS for Windows software, version 10.0 (SPSS, Chicago, Illinois). Significance levels were based on two-tailed tests, with α level set at .05.

Results

Demographic and medical characteristics

During the cardiac follow-up period, an early cardiac event occurred in 21 of 234 patients (9%), a late cardiac event occurred in 25 of 234 (11%) patients, and 188 of 234 patients (80%) had no cardiac event. Of the 25 late cardiac events 7 patients suffered from MI, 15 patients underwent re-PCI and 9 patients underwent CABG. Furthermore of these 25 patients 19 patients had 1 cardiac event, 5 patients had 2 cardiac events and one patient had 3 cardiac events after index PCI. Demographic and medical characteristics observed at baseline in the group with a late cardiac event and no cardiac event are presented in Table 1. No significant differences were found between these groups in demographic and medical characteristics, except for a higher diastolic blood pressure in the group with late cardiac events (p=0.01). More information about the PCI and coronary anatomy of the patients is presented in Table 2. Table 3 shows the untransformed data of the inflammatory markers at baseline. Distribution of inflammatory markers at 6 and 18 months were essentially the same, therefore only data from baseline are shown in Table 3.

Table 2. Coronary anatomy, result of PCI and re-angiography

2a

Coronary anatomy		Late event n=25	No event n=188	p
Single vessel angioplasty		22 (88%)	159 (88%)	0.65
Vessel	LAD	4 (18%)	49 (31%)	
	RCA	14 (63%)	72 (45%)	
	CX	1 (5%)	33 (20%)	
	GRAFT	3 (14%)	4 (3%)	
	LIMA		1 (1%)	
Stents implanted		16 (73%)	96 (60%)	0.26
Multi vessel angioplasty		3 (12%)	29 (12%)	0.65
Vessels*	LAD	5 (83%)	18 (30%)	
	RCA		28 (46%)	
	CX	1 (17%)	13 (21%)	
	GRAFT		2 (3%)	
Number of occlusions		6 (100%)	61 (100%)	
Stents implanted		3 (100%)	21 (72%)	0.29
Result of PCI**				
Unsuccessful >50%			1 (1%)	0.85
Successful				
Fair 20-50%		5 (20%)	45 (24%)	
Good <20%		20 (80%)	142 (75%)	

** Number of occlusions in the vessel between all patients undergoing multi vessel angioplasty*
***The result of PCI is the % of stenosis immediately after the PCI procedure*

2b

Re-angiography	76 (36%)
No change	48 (72%)
De novo lesion	3 (1%)
Restenosis	16 (27%)

Re-angiographies during the 24-month follow-up

Prediction of late cardiac events by inflammatory markers

High baseline concentrations of CRP (RR 2.5, 95%CI 1.1-5.7, p=0.04), IL-6 (RR 3.9, 95%CI 1.7-9.0, p<0.00) and IL-10 (RR 2.5, 95%CI 1.1-5.6, p=0.03) significantly raised the risk of a late cardiac event. High concentrations of IL-6 (RR 3.7, 95%CI 1.6-8.5, p<0.00) and IL-1ra (RR 2.6, 95%CI 1.1-6.1, p=0.04), measured at 6 months, significantly increased the risk of a late cardiac event (Table 4). None of the other markers of inflammation either at baseline or at 6 months increased the risk of a late cardiac event significantly. The inclusion of "intervention-no intervention" in the Cox proportional hazards analysis to control for the effect of the behavioral intervention, revealed essentially the same results.

Stability of inflammatory markers after PCI

Spearman rank correlations between baseline concentrations and 6 months after baseline show a high and strong stability in the synthesis of all markers except for IL-8. At 18 months, the correlation for IL-10, neopterin and IL-8 is weak. Stability for IL-6 is the strongest between both baseline-6 months and baseline-18 months. It must be noted that the 55 patients who where absent at 18 months due to loss-to-follow-up, had higher concentrations of IL-6 (Mann Whitney U:p<0.000) and IL-1ra (Mann Whitney U:p=0.014) at baseline. Therefore, the stability in synthesis of IL-6 and IL-1ra may have been underestimated. Taking the limitations into account, it is remarkable that the synthesis of the inflammatory markers after PCI is so stable.

Table 3. Untransformed data and distributions of immunological markers at baseline

	median	mean	sd	min*	max**
CRP (mg/l)	1.92	3.69	5.00	0.40	29.7
IL-6 (pg/ml)	1.72	2.01	0.93	0.82	5.41
TNF-α (pg/ml)	5.53	5.62	2.32	0.75	12.5
IL-10 (pg/ml)	2.30	3.06	2.06	2.30	28.9
IL-1ra (pg/ml)	184.1	231.7	222.8	79.4	1363
IL-8 (pg/ml)	26.1	90.0	156.3	3.80	836.0
neopterin (nmol/l)	6.55	6.95	3.83	0.70	19.0

**minimum*
***maximum*

Discussion

Of all inflammatory markers investigated in the current study, IL-6 is the strongest and most consistent predictor for the development of late cardiac events in exhausted PCI patients. A prognostic role of IL-6 with regard to the development of CAD was previously reported in both healthy and cardiac populations [11, 12], and may be related to its wide range of actions, including procoagulant effects on platelets, release of adhesion molecules by the endothelium, and the hepatic release of fibrinogen [21]. Furthermore, high concentrations of IL-6 are strikingly stable over a period of 18 months, suggesting a chronic involvement of IL-6 in the development of late cardiac events in exhausted PCI patients.

Table 4. Prediction of late cardiac events by inflammatory markers

	Late cardiac events*					
	Inflammatory markers measured at baseline			Inflammatory markers measured at 6 months		
	RR	95% CI	p	RR	95% CI	p
CRP	2.5	1.1-5.7	0.04	1.2	0.5-2.7	0.71
age	1.0	.95-1.1	0.89	1.0	.96-1.1	0.65
gender	.61	.24-1.6	0.30	.45	.18-1.2	0.10
stent	1.9	.74-4.9	0.18	1.7	.65-4.4	0.28
diabetes	2.4	.30-19	0.41	2.7	.34-22	0.34
BMI	1.0	.90-1.1	0.98	1.0	.94-1.2	0.48
current smoking	1.2	.44-3.4	0.69	.97	.34-2.7	0.95
diastolic bp	1.1	1.0-1.1	0.06	1.1	1.0-1.2	0.03
systolic bp	.99	.95-1.0	0.50	.99	.95-1.0	0.43
IL-6	3.9	1.7-9.0	0.00	3.7	1.6-8.5	0.00
age	1.0	.97-1.1	0.37	1.0	.96-1.1	0.47
gender	.63	.25-1.6	0.34	.54	.20-1.4	0.21
stent	2.5	.94-6.6	0.07	2.2	.83-5.9	0.11
diabetes	2.0	.25-16	0.52	2.3	.28-19	0.43
BMI	1.0	.92-1.1	0.71	1.0	.93-1.1	0.56
current smoking	.98	.36-2.7	0.97	.94	.33-2.7	0.92
diastolic bp	1.1	1.0-1.1	0.05	1.1	1.0-1.2	0.03
systolic bp	.98	.95-1.0	0.35	.98	.94-1.0	0.34
TNF-α	1.0	.38-2.7	0.99	.91	.34-2.5	0.86
age	1.0	.96-1.1	0.55	1.0	.96-1.1	0.60
gender	.53	.21-1.4	0.19	.46	.18-1.2	0.11
stent	1.8	.71-4.8	0.21	1.7	.65-4.4	0.29
diabetes	2.5	.32-20	0.37	2.7	.33-21	0.36
BMI	1.0	.93-1.1	0.58	1.0	.94-1.2	0.46
current smoking	.94	.35-2.5	0.90	.95	.34-2.7	0.93
diastolic bp	1.1	1.0-1.1	0.05	1.1	1.0-1.2	0.03
systolic bp	.99	.95-1.0	0.48	.99	.95-1.0	0.43
IL-10	2.5	1.1-5.6	0.03	1.2	.48-3.2	0.66
age	1.0	.97-1.1	0.45	1.0	.96-1.1	0.62
gender	.52	.20-1.3	0.16	.45	.18-1.2	0.10
stent	1.7	.65-4.3	0.29	1.7	.65-4.4	0.29
diabetes	2.0	.26-16	0.51	2.6	.33-21	0.36
BMI	1.0	.91-1.1	0.87	1.0	.93-1.2	0.49
current smoking	.81	.31-2.2	0.68	.96	.34-2.7	0.94
diastolic bp	1.1	1.0-1.1	0.04	1.1	1.0-1.2	0.03
systolic bp	.99	.95-1.0	0.42	.99	.95-1.0	0.42
IL-1ra	1.0	.39-2.7	0.96	2.6	1.1-6.1	0.04
age	1.0	.96-1.1	0.55	1.0	.96-1.1	0.54
gender	.53	.20-1.4	0.20	.55	.21-1.4	0.22
stent	1.8	.72-4.7	0.21	1.7	.67-4.6	0.26
diabetes	2.5	.32-20	0.37	3.4	.44-27	0.24
BMI	1.0	.93-1.1	0.58	1.0	.91-1.1	0.83
current smoking	.94	.35-2.5	0.91	.86	.31-2.4	0.78
diastolic bp	1.1	1.0-1.1	0.05	1.1	1.0-1.2	0.02
systolic bp	.99	.95-1.0	0.48	.98	.95-1.0	0.36

** 25 late cardiac events occurred, 188 patients had no recurrent cardiac event during the cardiac follow-up*

High CRP concentrations at baseline increase the risk of late cardiac events. CRP was previously shown to increase the risk of future cardiac events, both CRP concentrations that elicit a variety of proatherogenic effects in vascular tissue are typically in the range of 5-900 mg/l[24, 25]. Those concentrations are higher than the CRP concentrations that are used for clinical risk prediction (cut-off value 3 mg/l) and indicate that the precise role of CRP in the development of late cardiac events remains to be elucidated.

Little information is available regarding the potential role of anti-inflammatory markers in CAD. In the present study, high concentrations of the anti-inflammatory markers IL-1ra and IL-10 were found to increase the risk of late cardiac events. Circulating concentrations of the anti-inflammatory marker IL-1ra directly reflect the local inflammatory response of IL-1β at the site of atherosclerotic plaques [26]. Systemic measurement of the pro-inflammatory marker IL-1β proved to be impossible

Table 4. Prediction of late cardiac events by inflammatory markers (Continued)

	Late cardiac events*					
	Inflammatory markers measured at baseline			Inflammatory markers measured at 6 months		
	RR	95% CI	p	RR	95% CI	p
IL-8	.64	.22-1.9	0.42	.67	.22-2.0	0.48
age	1.0	.96-1.1	0.58	1.0	.96-1.1	0.69
gender	.55	.22-1.4	0.21	.47	.18-1.2	0.12
stent	1.9	.74-5.0	0.18	1.7	.66-4.5	0.26
diabetes	2.5	.33-20	0.37	2.8	.34-23	0.34
BMI	1.0	.92-1.1	0.66	1.0	.94-1.2	0.41
current smoking	.96	.36-2.6	0.94	.99	.35-2.8	0.98
diastolic bp	1.1	1.0-1.1	0.05	1.1	1.0-1.1	0.03
systolic bp	.99	.95-1.0	0.49	.99	.95-1.0	0.43
neopterin	2.0	.78-5.0	0.16	2.0	.71-5.4	0.19
age	1.0	.96-1.1	0.60	1.0	.96-1.1	0.69
gender	.54	.21-1.4	0.20	.45	.17-1.2	0.10
stent	1.7	.66-4.4	0.27	1.7	.65-4.4	0.28
diabetes	2.3	.30-18	0.42	2.8	.35-23	0.33
BMI	1.0	.93-1.1	0.58	1.0	.94-1.2	0.40
current smoking	.83	.30-2.3	0.71	.97	.35-2.7	0.96
diastolic bp	1.1	1.0-1.2	0.03	1.1	1.0-1.2	0.02
systolic bp	.99	.95-1.0	0.40	.98	.95-1.0	0.36

** 25 late cardiac events occurred, 188 patients had no recurrent cardiac event during the cardiac follow-up*

in the current patient population, probably due to the short half-life of IL-1β in blood of approximately 20 minutes [27]. The increased risk of late cardiac events associated with IL-1ra suggests that the reflection of IL-1β by IL-1ra identifies patients who are more prone to develop these events after PCI.

It is more difficult to understand why elevated concentrations of IL-10 increase the risk of a late cardiac event in exhausted PCI patients. The focus of earlier studies on the association of low concentrations of IL-10 with CAD hampers a direct comparison of current and previous findings. Lower concentrations of IL-10 were found in unstable angina (UA) patients who suffered from future cardiac events as opposed to UA patients without those events [28]. Serum concentrations of IL-10 were also lower in UA patients compared to patients with stable angina [29] or MI [30], indicating different patterns of inflammatory reactions in the pathophysiology of CAD in these clinical conditions. One study however found that acute coronary syndrome patients with high concentrations of IL-10 were at significantly lower risk than patients with lower IL-10 concentrations [31]. The follow-up was only three months, these cardiac events are therefore different from cardiac events described in the current study. The increased risk of late cardiac events associated with high concentrations of IL-10 suggests that maximal contra regulatory mechanisms are activated, but fail in their mission.

Limitations

The major limitation of the present study is the limited external validity. Only patients who felt exhausted after PCI were included. Furthermore, patients were included approximately one month after index PCI. Thus, although an unknown number of early events directly after PCI were missed, it can be assumed that all events due to inadequate PCI were excluded from the current study.

Table 5. Stability of inflammatory markers after PCI

baseline	6 months n=226	18 months n=179
CRP	0.67*	0.67*
IL-6	0.94*	0.89*
TNF-α	0.78*	0.68*
IL-10	0.46*	0.30*
IL-1ra	0.55*	0.63*
IL-8	0.24*	-0.04
neopterin	0.44*	0.26*

**Correlation is significant at the .01 level (2-tailed)*

Earlier studies observed a positive association between exhaustion and IL-1β and IL-6 [6,7]. Thus, the concentration of the inflammatory markers in the present study may have been somewhat higher compared with those in an unselected group of PCI patients. The behavioral intervention reduced the risk of a late cardiac event by 21% in the study sample. Although this reduction was not significant (RR 0.79, 95%CI .4-1.8, p=0.56), it cannot be ruled out that it may have resulted in an underestimation of the relative risk of late cardiac events associated with increased concentrations of inflammatory markers. However, the inclusion of "intervention-no intervention" in the Cox proportional hazards analysis to control for the effect of the behavioral intervention on the association between markers of inflammation and late cardiac events revealed essentially the same results.

Due to the design of the study a blood sample before the index PCI was lacking. However, increases in inflammatory markers after PCI are transient, return to pre-procedural concentrations within a week and remain at pre-procedural concentrations up to at least one month after PCI. It can therefore be argued that post-procedural concentrations of inflammatory markers, measured in blood approximately 6 weeks after index PCI, reflect the inflammatory status before the PCI because this procedure does not remove the inflammatory lesion. The current results on temporal stability over 18 months of the inflammatory markers only strengthen this suggestion, even though the measurement of 18 months after baseline only comprised 179 patients. However, the loss-to-follow-up of 55 patients most likely led to an underestimation of the stability for IL-6 and IL-1ra. Finally, although the relative risk for late cardiac events may be different for various indications for PCI, the number of late cardiac events was not sufficient to stratify for "indication for PCI" and analyze for late cardiac events.

Conclusions

High concentrations of CRP, IL-6, IL10 and IL-1ra increase the risk of late cardiac events in exhausted PCI patients. IL-6 synthesis is chronically increased after PCI. This study supports the assumption that inflammation is a pathophysiological mechanism in the development of CAD.

Acknowledgements

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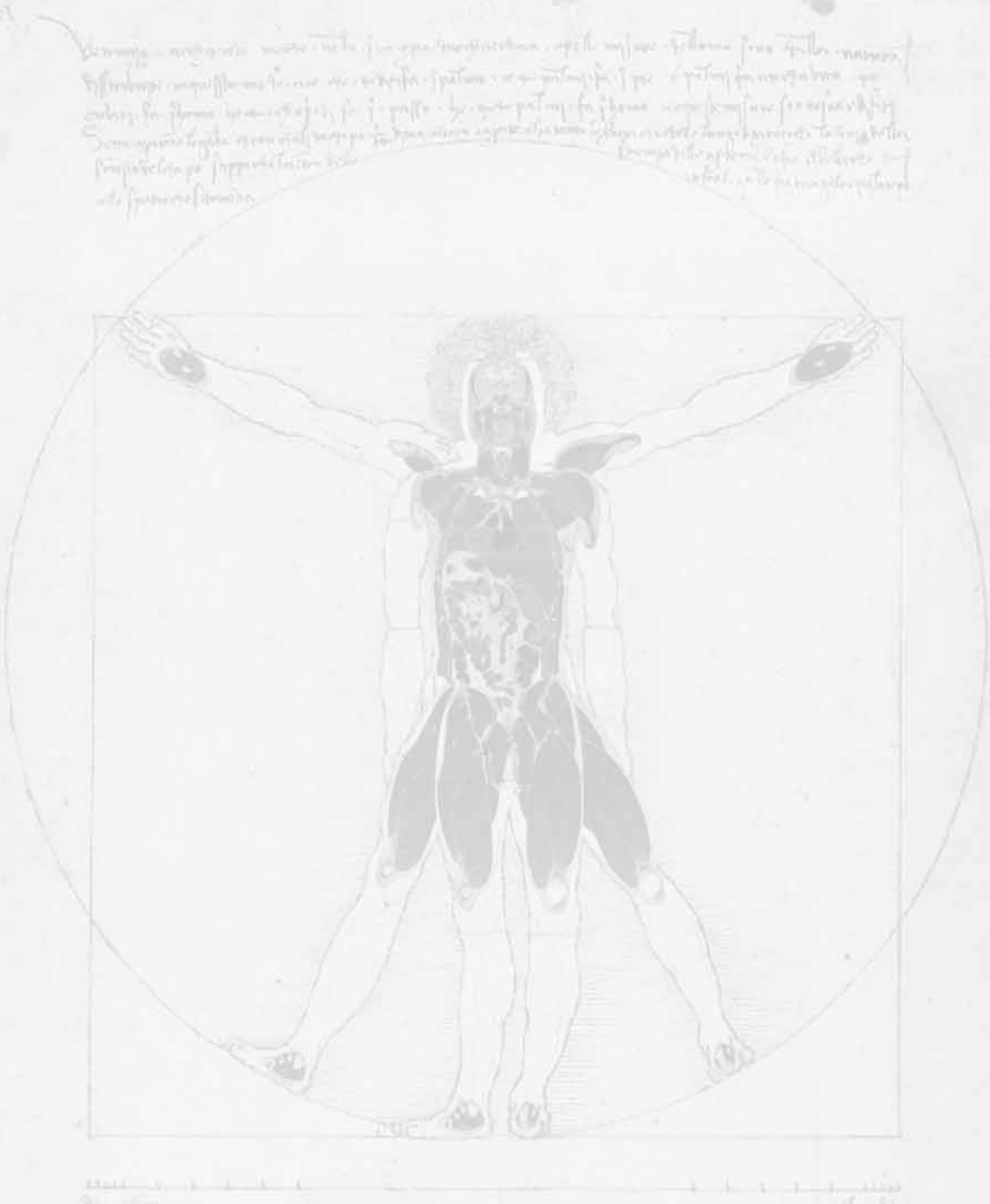
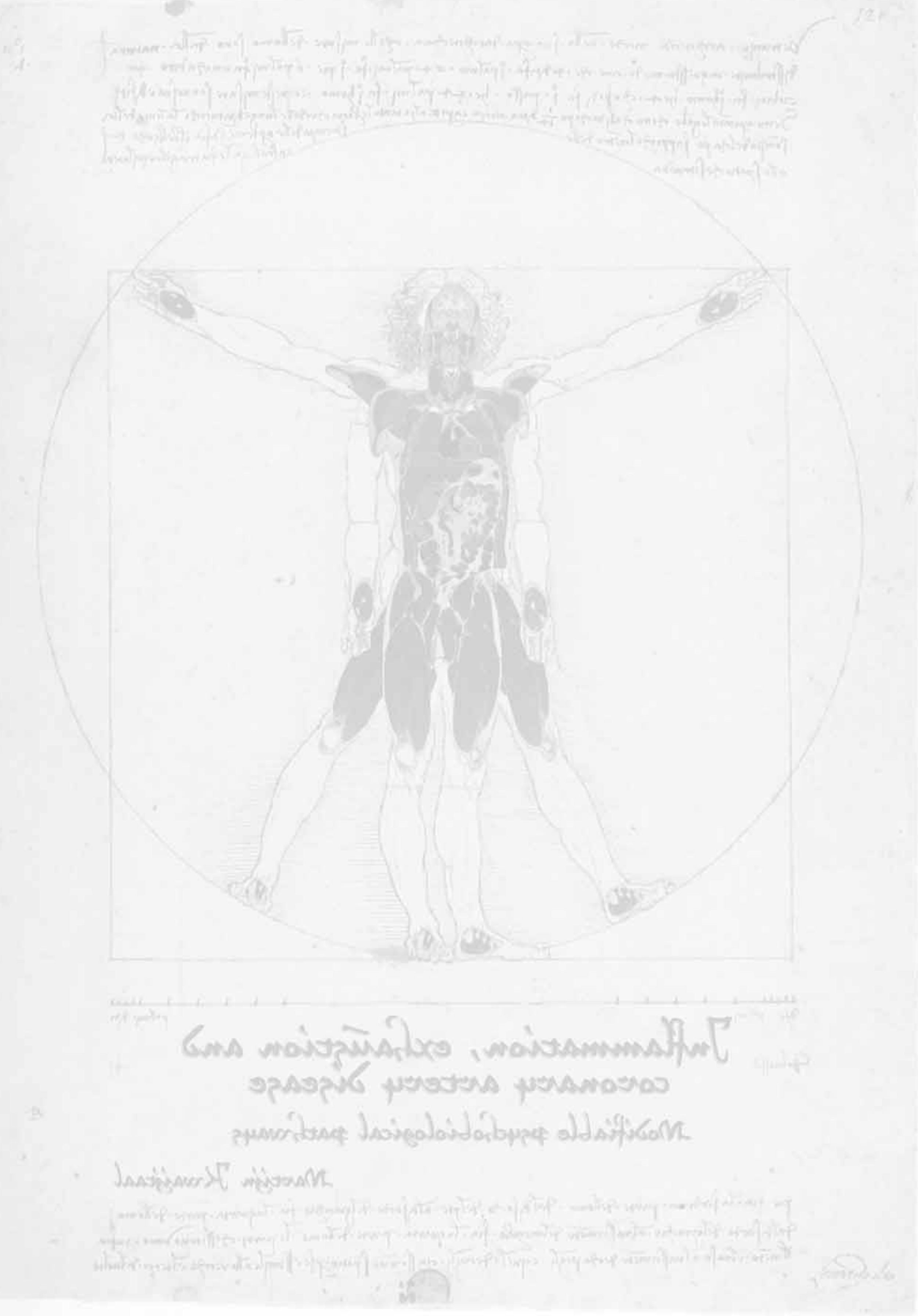


Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

Martijn Kwaijtaal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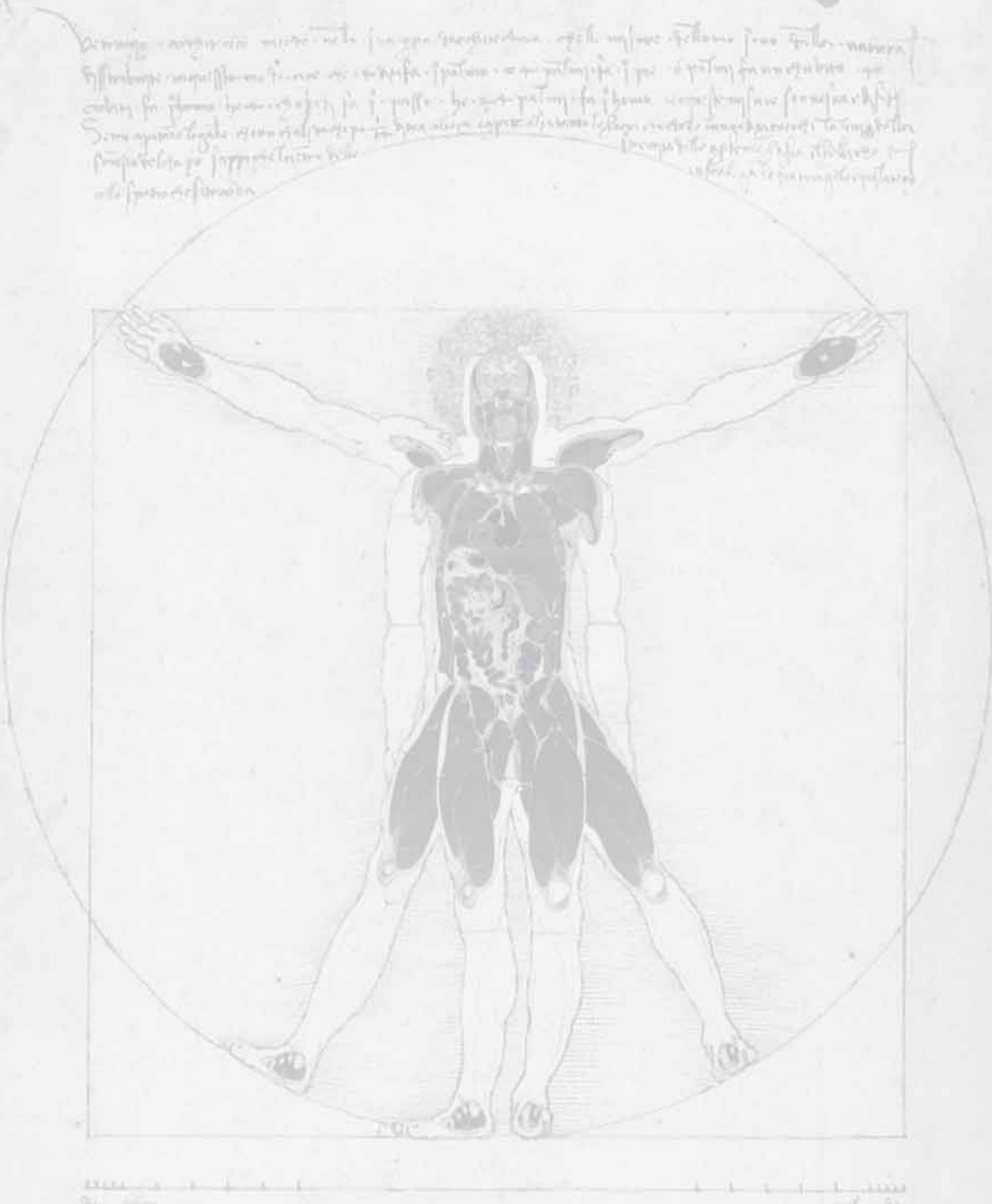


Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

Martijn Kraaijtaal

3

A behavioral intervention reduces inflammation through activating vagal activity

Part I: Effect of the behavioral intervention on inflammation

Martijn Kwaijtaal, André J. van der Ven, Rob van Diest, Frits W. Bär, Cathrien A. Bruggeman, Marc H. de Baets, Ad Appels

Submitted

Abstract

Aims: Atherosclerosis is an inflammatory disease. The inflammatory response is controlled by the neurohormonal system. We tested the hypothesis that a behavioral intervention aimed at the reduction of distress, approached as a state of exhaustion, reduces inflammation.
Methods and Results: The present study is a sub-study of the EXhaustion Intervention Trial, a randomized controlled trial designed to test the hypothesis that intervening on exhaustion reduces the risk of a new coronary event in patients who felt exhausted after percutaneous coronary intervention. The intervention was based on group therapy focusing on stressors leading to exhaustion, and on support for recovery by promoting rest and making rest more efficient. Blood samples were drawn at baseline, after six months and after 18 months in 111 participants and 123 controls. The samples were analyzed for neopterin, interleukin 1 receptor antagonist (IL-1ra) and tumor necrosis factor (TNF)-α. These cytokines are markers of monocyte-macrophage activation. The intervention reduced the odds of elevated neopterin by 68% (OR=0.32; 95%CI .13-.83; p=0.02), and the odds of elevated IL-1ra by 67% (OR=0.33; 95%CI -.13-.72; p=0.01) in those who did not suffer from co-morbidity. The intervention had no effect on TNF-α.
Conclusions: The intervention reduced the odds of elevated concentrations of neopterin and IL-1ra at 18 months. A behavioral intervention aimed at the reduction of a state that activates immune-mediated inflammation contributes to the control of the inflammatory response.

Key words: *angioplasty, behavioral intervention, inflammation, exhaustion*

Introduction

Inflammation is a protective response to microbial invasion or tissue injury. The initial response is formed by the release of pro-inflammatory cytokines. This release is followed by the release of anti-inflammatory mediators to maintain homeostasis. Loss of endogenous anti-inflammatory mechanisms converts a normally self-limited inflammatory response into an excessive response that is potentially deleterious [1].

The inflammatory response is modulated by the nervous system in two ways. Inflammatory stimuli activate sensory pathways that relay information to the hypothalamus that activates an anti-inflammatory response. Efferent activity of the nervus vagus leads to acetylcholine release that deactivates the macrophages by inhibiting the release of tumour necrosis factor-a (TNF-α), interleukin-1β (IL-1β) and other cytokines (the "inflammatory reflex") [2]. The other pathway includes the hypothalamus-pituitary-adrenocortical (HPA) axis that produces cortisol. High levels of cortisol suppress inflammation.

The modulation of inflammation by neural pathways made us wonder whether a behavioral intervention may contribute to a decrease of inflammation in coronary artery disease (CAD) patients. More specifically we tested the hypothesis that the behavioral intervention decreases the number of patients with elevated concentrations of IL-1ra, neopterin and TNF-α. These cytokines are markers of monocyte-macrophage activation [3, 4]. Monocyte-macrophage activation plays a central role in the development and progression of atherosclerosis [1].

We selected exhausted patients because a state of exhaustion has been found to be positively associated with markers of inflammation and negatively with the adenocorticotrope hormone (ACTH), cortisol and glucocorticoid sensitivity [5-9]. This suggests that exhausted subjects are characterized by a decreased activity of the HPA axis. This pro-inflammatory state may be one of the pathophysiological mechanisms that underlies the association between a state of exhaustion and CAD observed in epidemiological studies [10-12].

Methods

Patient selection

This immunological study is a sub-study of the EXhaustion Intervention Trial (EXIT), a randomized controlled trial designed to test the hypothesis that a behavioral intervention on exhaustion in percutaneous coronary intervention (PCI) patients reduces the risk of a new cardiac event [13]. Briefly, participants of EXIT were 710 patients, aged 35-68 years, who felt exhausted after successful PCI. Exhaustion was measured in two phases. First, two weeks after PCI, patients completed the Maastricht Questionnaire (MQ; range=0-46), a standardized self-administered instrument designed to measure exhaustion [14]. Patients with a MQ score of 14 and higher entered the second phase, which consisted of the Maastricht Interview for Vital Exhaustion (MIVE; range=0-23). MIVE has a higher predictive validity for future cardiac events than the MQ [15]. Patients who felt exhausted after PCI, as indicated by a score ≥7 on the MIVE were included.

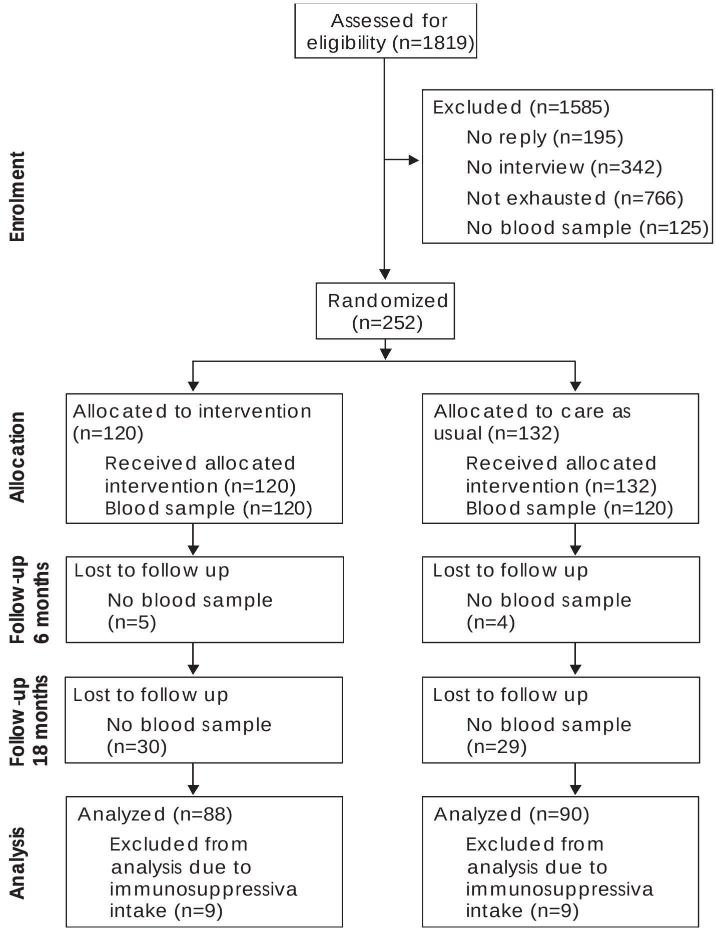
Major exclusion criteria were: 1) severe somatic or mental comorbidity (e.g. renal insufficiency, a history of major depression of at least 3 years), 2) somatisation disorder, fibromyalgia or chronic fatigue 3) participation in a behavioral rehabilitation program other than EXIT, 4) unsuccessful treatment for a recent mood or anxiety disorder, and 5) inability to speak Dutch. Thus, only patients who fulfilled strict criteria for exhaustion were included in the study. In the context of the immunological study to be reported below it is important to note that none of the patients reported to be treated for a chronic viral infection such as HCV or HIV in the year before PCI. Patients were randomized in an intervention group and a control group. In the control group patients received care as usual. The median interval between PCI and inclusion in the study was 27 days. The institutional review board of the participating center approved the study protocol and all participants gave written informed consent.

Behavioral intervention

The association between the mental state of coronary patients and inflammation is bi-directional. Prolonged stress may result in feelings of exhaustion and a decreased activity of the HPA axis that activates immune-mediated inflammation. Inflammation may cause feelings of tiredness and malaise as part of the inflammatory reflex. Thus the nature of the feelings of exhaustion, experienced by more than half of all PCI patients, is ambiguous. They may reflect a breakdown in adaptation to prolonged stress and form part of a healthy reaction to inflammation. Therefore the intervention was directed at coping with the stressors leading to exhaustion and at accepting tiredness as a signal that the body needs rest.

Group discussions about the (mostly idiosyncratic) stressors that supposedly had caused exhaustion were the main tool to help patients to cope with these stressors. This part of the intervention was supported by hostility therapy, because anger and hostility belong to the causes of exhaustion. We used methods developed by Williams & Williams and by Powel to treat hostility [16, 17]. Recovery was promoted by discussing the minimum and maximum length of resting time, by relaxation exercises designed to reduce tension and to make rest more efficient [18], by stimulating physical exercise, and by assigning homework. If, for example, two or more patients felt very tired at noon but were reluctant to slow down, these patients were invited to form a “siesta group” and to phone each other at night to discuss whether or not they had taken a nap. The intervention consisted of 10 weekly sessions with six patients, followed by four monthly sessions. Sessions lasted two hours and partners were encouraged to attend the meetings.

Figure 1. CONSORT flow diagram of the immunological sub-study



Blood collection

For the present study, blood samples were collected in one participating center (Maastricht). Baseline blood samples were collected of 252 consecutive patients approximately two weeks after inclusion. A medical specialist (second author) screened the medical records of these patients for the use of immunosuppressive medication. This resulted in the exclusion of 18 patients. So, baseline data were available for 234 patients. Follow-up samples at 6 months after baseline were collected in 226 patients, 8 patients were missed due to loss-to-follow-up (e.g. patients did not show up, mistakes in lab while processing blood samples, death, patients refused to give blood). At 18 months blood was collected of 181 patients, 53 patients were missed due to loss-to-follow-up (Figure 1). Blood was allowed to clot at room temperature for 30 minutes and centrifuged. Serum samples were stored at -20°C in anticipation of further processing.

Immunological assessments

IL-1 receptor antagonist

As a measure of monocyte and macrophage activation IL-1 receptor antagonist (IL-1ra) was analyzed. IL-1ra was measured in serum in duplicate using quantitative enzyme-linked immunosorbent assay (ELISA). The ELISA-kit was purchased from DiaMed Eurogen (Turnhout, Belgium). Test samples and standard solutions were incubated on pre-coated ELISA plates. A biotinylated secondary antibody against IL-1ra was used. Peroxydase conjugated streptavidin was then applied to bind to the biotinylated antibody and after washing, a tetramethylbenzidine-solution (TMB) was used to stain the remainder of the bound streptavidin; the reaction was stopped by adding sulfuric acid.

Optical densities were read using a PowerWaveX Reader (MWG Biotech, Ebersberg, Germany), data was calculated using KC4 software (MWG Biotech, Ebersberg, Germany). Sensitivity of all tests was calculated by the mean of 6 zero-values +3 standard deviations extrapolated on the standard curve. All samples that were below the sensitivity value were discarded and replaced with the value of sensitivity of the corresponding assay. Intra-assay and inter-assay coefficient of variation (CV) was 6.1% (n=10), and 9.2% (n=7), respectively.

Neopterin

Neopterin was also measured in duplicate as a marker of monocyte and macrophage activation using a competitive ELISA (IBL-Hamburg, Hamburg, Germany) in accordance with the manufacturer's recommendations. Intra-assay and inter-assay CV was 3.6% (n=11) and 7.6% (n=6), respectively.

TNF-α

TNF-α was analyzed as a marker of inflammation using an ELISA purchased from DiaMed Eurogen. Test principles are described in the IL-1ra section. Intra-assay and inter-assay CV was 5.8% (n=10) and 10.2% (n=7), respectively. Because not enough serum was available to analyze all markers in all patients, the number of missing concentrations was slightly larger for neopterin and IL-1ra.

Statistical analyses

To explore demographic and medical characteristics that may confound the association between inflammatory markers and the effect of the behavioral intervention χ^2 tests and t-tests were used to compare the intervention group and the control group. As missing data at 18 months may have affected the comparability of the intervention group and the control group obtained by randomization at baseline, we checked whether the demographic and medical characteristics of both groups observed at baseline differed at 18 months. Furthermore we checked whether missing data were associated with any of the baseline variables.

Because the inflammatory markers were not normally distributed, even after log transformation, the data were categorized into quartiles. Categorization is common in risk prediction for inflammatory markers [19-21]. We used a score in the highest quartile as defined by the distribution at baseline as index for inflammation. Top quartile groups were coded as 1 and the lower three quartiles were coded as 0.

This sub-study is a 'mechanistic' study that investigates the effect of a behavioral intervention on the biological mechanisms that may underlie the association between a putative risk factor and disease. Therefore, missing data were not imputed.

The effect of the intervention on markers of inflammation was tested by Cochran's Q test, and by logistic regression analyses. The Cochran's Q procedure tests the null hypothesis that multiple ordinal responses come from the same population. Because patients with any missing values are excluded in this analysis the numbers and percentages in Table 3 may differ slightly from those presented in Tables 1 and 2. In the logistic regression analyses a score in the upper quartile at 18 months (as defined by baseline concentrations), formed the dependent variable. Independent variables were treatment group, a score in the upper or lower quartiles at baseline (to control for initial concentrations), and age. Age was included as covariate because the intervention group was older than the control group and because age was positively associated with some markers of inflammation.

To investigate whether the effect of the intervention was modified by age, gender, a previous history of CAD, co-morbidity (defined as the presence of a chronic inflammatory or painful condition not meeting the exclusion criteria) or smoking, the logistic regression analyses were repeated to check for possible interactions. Data processing and statistical analysis was performed using SPSS for Windows software, version 10.0 (SPSS, Chicago, Illinois). Significance levels were based on two-tailed tests, with α level set at .05.

Results

Subjects

No difference in baseline characteristics between the intervention group and the control group was observed, except for age and diabetes. These factors approached statistical significance (Table 1) and were used as covariates in the multivariate analyses. Table 2 shows that loss-to-follow-up did not affect the comparability of both groups on baseline demographic and medical characteristics. Missing concentrations were equally distributed among the intervention group and the control group. However, missing concentrations were not randomly distributed among some relevant subgroups. Those who smoked at baseline had more missing concentrations at 18 months of neopterin and IL-1ra. Smokers also had higher neopterin concentrations at baseline. Women had more missing concentrations of neopterin and IL-1ra at 18 months. Women also had higher IL-1ra concentrations at baseline. Therefore, age, gender and smoking were included in the multivariate analyses of neopterin. Age and gender were included as possible confounders in the multivariate analysis of IL-1ra.

Table 1. Baseline characteristics of the intervention and control group

	Intervention n=111	Control n=123		p
Demographics			*t*	
Age	54.7 (±6.9)	52.9 (±7.7)	1,92	0.06
BMI	27.0 (±3.8)	27.2 (±4.1)	-0,32	0.75
Blood pressure				
Systolic	131.4 (±18.8)	132.1 (±18.4)	-0,27	0.78
Diastolic	82.4 (±9.9)	83.7 (±11.2)	-0,93	0.35
MIVE	13.1 (±4.3)	12.6 (±3.8)	0,91	0.36
Gender			*chi*	
Male	89 (80%)	91 (74%)	1,26	0.26
Female	22 (20%)	32 (26%)		
Smoking				
Current	21 (19%)	24 (19%)	0,05	0.98
Stopped	79 (71%)	86 (70%)		
Never	11 (10%)	13 (11%)		
Medical characteristics				
Diabetes	7 (6%)	17 (14%)	3,58	0.06
Major depression	22 (20%)	17 (14%)	1,51	0.22
Chronic painful condition	14 (13%)	9 (7%)	1,85	0.17
Cardiac history				
Previous MI	28 (25%)	31 (25%)	0,00	1.00
Previous CABG	11 (10%)	10 (8%)	0,23	0.63
Previous PCI	16 (14%)	15 (12%)	0,25	0.62
Indication for PCI				
Stable Angina	10 (9%)	13 (10%)	3,36	0.50
Unstable Angina	66 (59%)	70 (57%)		
Myocardial Infarction	24 (22%)	23 (19%)		
Post MI angina	11 (10%)	17 (14%)		
Stenoses after PCI (occlusion > 50%)				
0	51 (46%)	56 (46%)	1,48	0.69
1	34 (31%)	31 (25%)		
2 or more	26 (23%)	36 (29%)		
Stent implanted	77 (69%)	75 (61%)	1,81	0.18
Elevated concentrations of *				
neopterin	31 (28%)	27 (22%)	1,12	0.29
IL-1ra	27 (24%)	31 (25%)	0,02	0.88
TNF-alpha	25 (23%)	33 (25%)	0,58	0.45
Medication after PCI				
Ace inhibitor	30 (24%)	22 (20%)	0,71	0.40
Diuretics	13 (11%)	12(11%)	0,00	0.95
Beta-blocker	92 (75%)	88 (79%)	0,66	0.42
Calcium antagonists	51 (42%)	38 (34%)	1,29	0.26
Statins	92 (75%)	85 (77%)	0,10	0.75
Nitrates	98 (80%)	89 (80%)	0,01	0.92

** patients in highest quartile*
BMI = body mass index, MIVE = Maastricht interview for vital exhaustion, MI = myocardial infarction, CABG = coronary artery bypass graft surgery, PCI = percutaneous coronary intervention

Table 2. Demographic and medical characteristics *(assessed at baseline)* of the intervention and control group at 18 months

	Intervention n=88	Control n=90		p
Demographics			*t*	
Age	54.4 (±6.7)	53.7 (±7.5)	0,63	0.53
BMI	27.1 (±3.8)	27.4 (±4.1)	-0,49	0.62
Blood pressure				
Systolic	131.0 (±19.2)	132.9 (±18.7)	-0,68	0.50
Diastolic	83.7 (±8.6)	84.1 (±10.7)	-0,30	0.77
MIVE	13.4 (±4.3)	12.7 (±3.8)	0,96	0.34
Gender			*chi*	
Male	74 (84%)	70 (78%)	1,15	0.28
Female	14 (16%)	20 (20%)		
Smoking				
Current	14 (16%)	12 (13%)	0,38	0.83
Stopped	65 (74%)	70 (78%)		
Never	9 (10%)	8 (9%)		
Medical characteristics				
Diabetes	5 (6%)	14 (16%)	4,55	0.03
Major depression	15 (17%)	11 (12%)	0,93	0.36
Chronic painful condition	8 (9%)	6 (7%)	0,36	0.55
Cardiac history				
Previous MI	25 (28%)	21 (23%)	0,60	0.44
Previous CABG	9(10%)	8 (9%)	0,09	0.76
Previous PCI	12(14%)	11(12%)	0,08	0.78
Indication for PCI				
Stable Angina	6 (7%)	7 (8%)	1,75	0.78
Unstable Angina	51 (58%)	55 (61%)		
Myocardial Infarction	22 (25%)	18 (10%)		
Post MI angina	9 (10%)	10 (11%)		
Stenoses after PCI				
0	40 (46%)	42 (47%)	0,82	0.85
1	25 (28%)	21 (23%)		
2 or more	23 (26%)	27 (30%)		
Stent implanted	63 (72%)	60 (67%)	0,51	0.48
Elevated concentrations of *				
neopterin	11 (13%)	18 (21%)	1,57	0.21
IL-1ra	19 (22%)	23 (26%)	0,34	0.56
TNF-alpha	20 (23%)	32 (36%)	3,37	0.07
Medication after PCI				
Ace inhibitor	19 (22%)	22 (24%)	0.20	0,65
Diuretics	7 (8%)	10 (11%)	0.51	0,47
Beta-blocker	69 (78%)	66 (73%)	0.63	0,43
Calcium antagonists	30 (34%)	33 (37%)	0.13	0,72
Statins	74 (84%)	71 (79%)	0.80	0,37
Nitrates	73 (83%)	72 (80%)	0.26	0,61

** patients in highest quartile*
BMI = body mass index, MIVE = Maastricht interview for vital exhaustion, MI = myocardial infarction, CABG = coronary artery bypass graft surgery, PCI = percutaneous coronary intervention

We also controlled for diabetes, because the prevalence of diabetes was higher in the control group than in the intervention group. Controlling for diabetes revealed essentially the same results, because diabetes was not associated with any of the markers of inflammation. Therefore results of analyses controlling for diabetes will not be reported below.

Effect of the intervention on markers of inflammation

Neopterin

Neopterin concentrations ranged from 0.70-9.18 nmol/l in the three lowest quartiles and from 9.19-19.0 in the upper quartile. The percentage of patients with elevated neopterin concentrations declined significantly in the intervention group. No decline in the control group was observed (Table 3).

Table 3. Test for linearity over time in highest quartiles in the intervention and control group

	Intervention	Q	df	p	Control	Q	df	p
Neopterin	n=83				n=87			
baseline	22 (27%)	12,54	2	0,00	21 (24%)	0,51	2	0.77
6 months	11 (13%)				18 (21%)			
18 months	8 (10%)				18 (21%)			
IL-1ra	n=82	0,29	2	0,87	n=87	3,31	2	0,19
baseline	17 (22%)				22 (25%)			
6 months	18 (22%)				26 (30%)			
18 months	16 (20%)				30 (34%)			
TNF-alpha	n=83	0,47	2	0,79	n=88	1,87	2	0,39
baseline	19 (23%)				31 (35%)			
6 months	21 (25%)				33 (38%)			
18 months	21 (25%)				27 (31%)			

Consequently the number of patients with elevated concentrations of neopterin at 18 months was significantly smaller in the intervention group (χ^2=5.00; p=0.03). No effect modifiers were detected. Results of the logistic regression analysis showed that the intervention reduced the odds of being in the upper quartile at 18 months by 68% (OR=0.32; 95%CI .13-.83; p=0.02), controlling for age, gender, smoking and neopterin concentration at baseline.

IL-1ra

IL-1ra concentrations ranged from 49-270 pg/ml in the three lowest quartiles and from 271-1361 pg/ml in the upper quartile. No significant changes in the percentage of patients with elevated IL-1ra were observed in the intervention group or in the control group. However, the percentage tended to decrease in the intervention group and to increase in the control group. At 18 months, 21% of the patients in the intervention group and 36% of the patients in the control group had elevated concentrations of IL-1ra (χ^2 = 4.82; p=0.03) (Table 3). The effect of the intervention was modified by 'co-morbidity'. The intervention reduced the odds of having an elevated concentration of IL-1ra at 18 months by 67% in those who did not suffer from a chronic inflammatory or painful condition (OR=0.33; 95%CI .13-.72; p=0.01), controlling for age, gender and IL-1ra concentrations at baseline. In contrast the intervention had no effect on IL-1ra in those who suffered from a chronic inflammatory or painful condition. Thus the data suggest that the intervention had a beneficial effect on the natural course of IL-1ra in those who did not suffer from co-morbidity. Most diseases classified as co-morbidity were inflammatory diseases that sustain elevated concentrations of IL-1ra.

TNF-α

There was no sign of significant changes in the percentage of patients with elevated concentrations of TNF-α within the groups. No effect modifiers were detected. The intervention did not change the odds of having elevated concentrations of TNF-α at 18 months (OR=1.17; 95%CI .56-2.42; p=0.68).

Discussion

The behavioral intervention used in the EXIT study decreased the number of patients with elevated concentrations of neopterin and IL-1ra. These are cytokines produced by macrophages as an initial response to inflammation. Excessive or prolonged response to injury of a vessel wall contributes to atherosclerosis. Thus, the behavioral intervention contributed to the control of the inflammatory response.

The effect of the intervention on the natural course of IL-1ra was modified by “co-morbidity”. Nearly all patients classified with a co-morbid condition suffered from a chronic inflammatory condition (e.g. Rheumatoid Arthritis, Bechterev, Crohn’s disease). These patients did not match the additional exclusion criterion for immunosuppressive medication intake and were therefore included in the sub-study. “Co-morbidity” was also found to increase the risk of late coronary events [13]. The intervention had no effect on TNF-α levels.

The statistical analyses were restricted to available cases because this study was not designed to test the hypothesis that the behavioral treatment used in this study is superior to other methods to treat inflammation, and because imputing missing data may invalidate a mechanistic model by the artificial increase of the stability of the study factor. This approach is open for discussion. Therefore the analyses were repeated replacing missing values by the last observed value. Results showed that the number of patients with elevated levels of neopterin decreased significantly in the intervention group and not significantly in the control group. The multivariate analyses showed that the intervention reduced the odds of having an elevated level of neopterin by 54% (OR=0.46; 95%CI .21-1.03; p=0.06) and an elevated level of IL-1ra by 56% (OR=0.44; 95%CI .20-.10; p=0.04). Thus, an analysis of the data according to intention-to-treat principles gives essentially the same results.

It is not generally accepted that the mental state of coronary patients can be best described as a state of exhaustion. Many scientists prefer to approach the symptoms of lack of energy, increased irritability and general malaise as a clinical depression, i.e. as a mental disorder. In this study 39 patients (17%) met DSM IV criteria for Major Depression. One might argue that the behavioral intervention reduced elevated levels of neopterin and IL-1ra because it reduced the number of patients suffering from Major Depression. Therefore the analyses were repeated excluding patients who were depressed at baseline. Results showed that the intervention reduced the odds of elevated neopterin by 78% (OR=0.22; 95%CI .07-.65; p=0.01), and the odds of elevated IL-1ra (in those without co-morbidity) by 67% (OR=0.33; 95%CI .14-.78; p=0.01) in exhausted non-depressed patients. These results show that coronary patients who manifest many symptoms of a pro-inflammatory state without being depressed may profit from a behavioral intervention.

In addition to TNF-α, IL-1ra and neopterin other markers of inflammation were also measured (i.e. IL-6, CRP, IL-10 and IL-8). Because this study focuses on monocyte-macrophage activation, only TNF-α, IL-1ra and neopterin were reported. No changes were found in patients with high concentrations of CRP, IL-10, IL-8 or IL-6 over 18 months of follow-up.

Conclusions

A behavioral intervention aimed at the reduction of a state that activates immune-mediated inflammation contributes to the control of the inflammatory response. We hypothesize that the beneficial behavioral intervention effects on inflammation were mediated by increased nervus vagus activity. This will be investigated in part II of this article.

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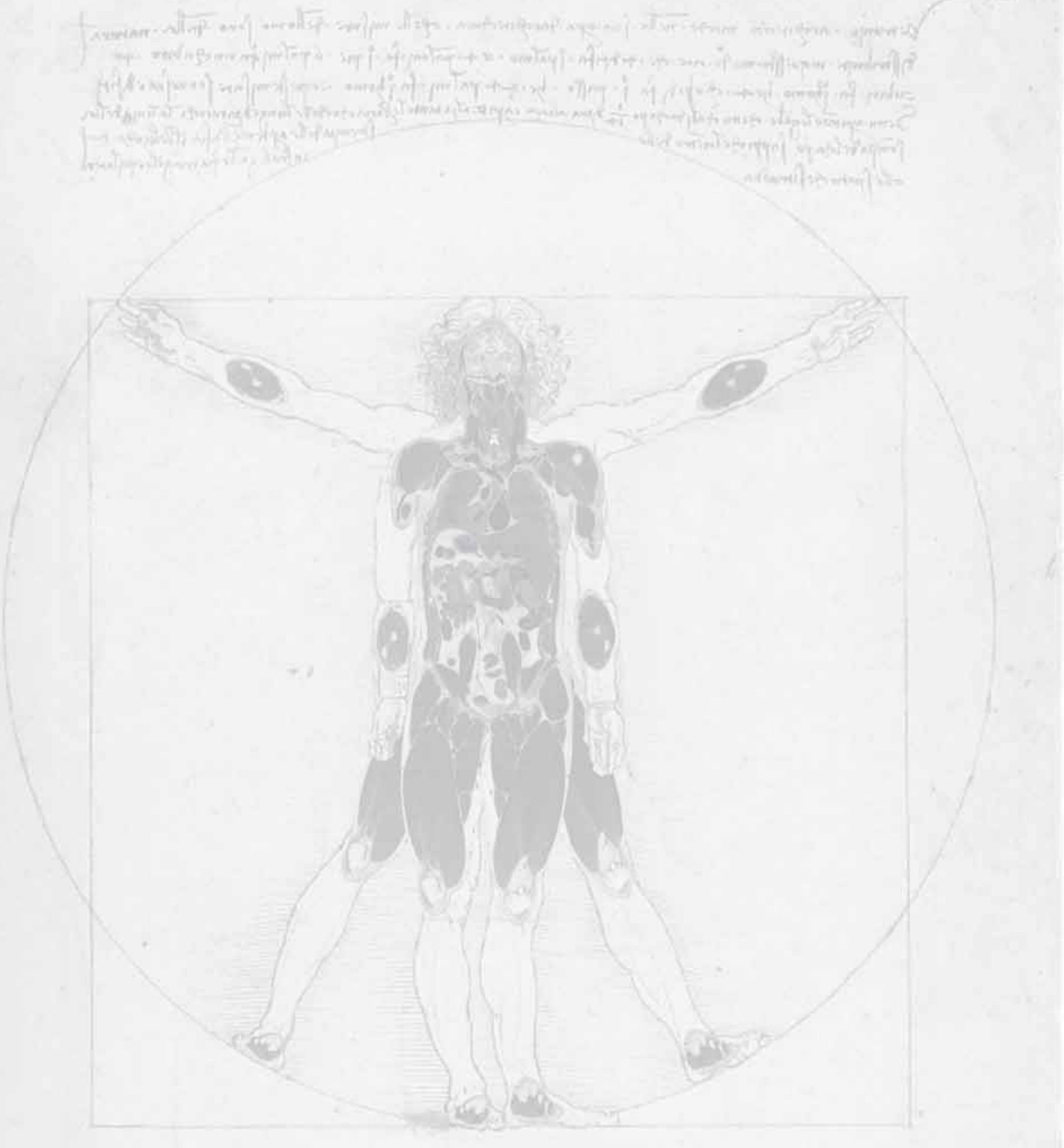
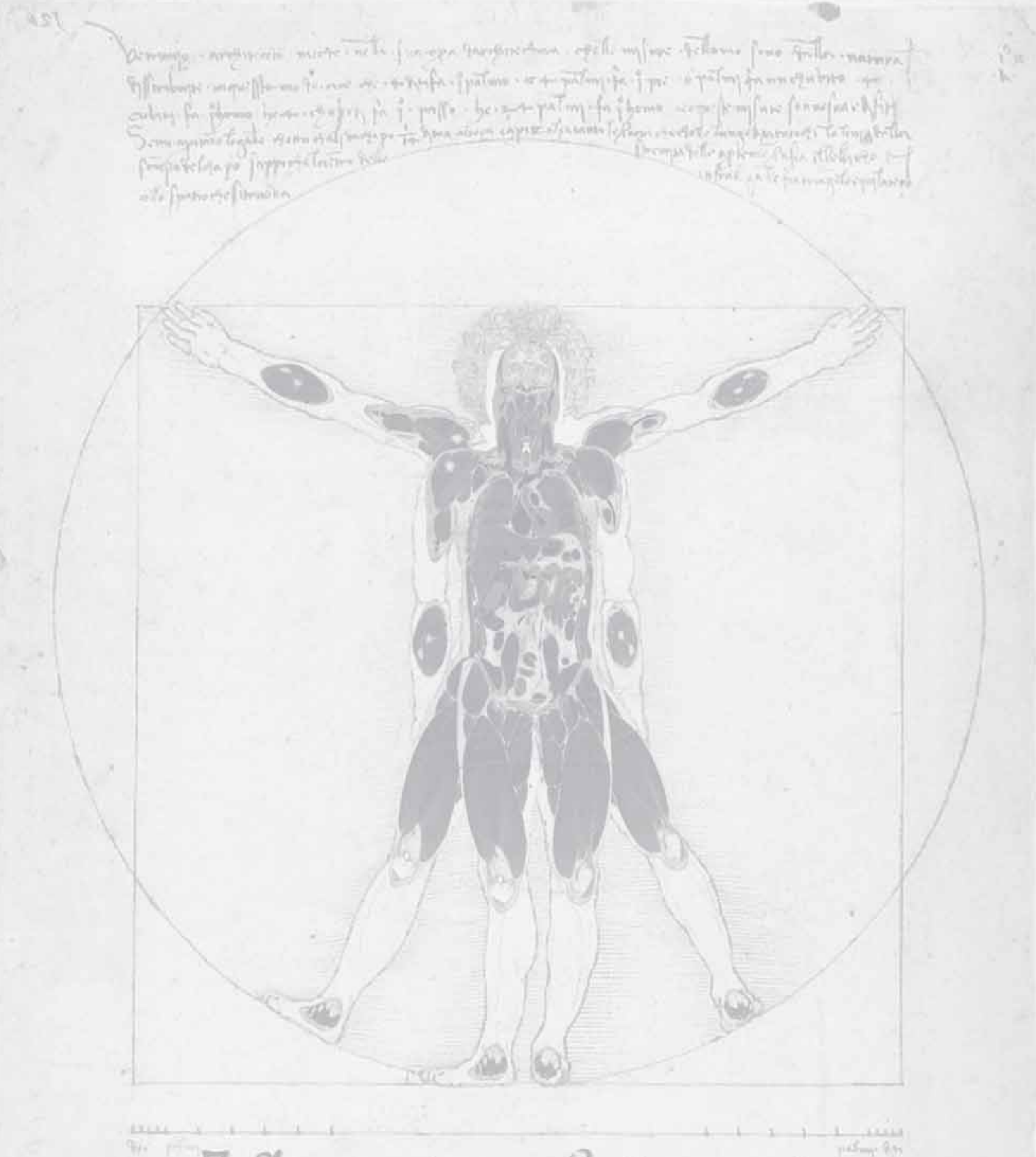


Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

Martijn Kwaijtaal



Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

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4

A behavioral intervention reduces inflammation through activating vagal activity

Part II: The mediating role of the nervus vagus

Martijn Kwaijtaal, André J. van der Ven, Rob van Diest, Frits W. Bär, Cathrien A. Bruggeman, Marc H. de Baets, Ad Appels

Submitted

Abstract

Aims: The study in part I of this article showed that inflammation can be modified by a behavioral intervention. The hypothesis addressed in this part of the study is that the beneficial behavioral intervention effects on inflammation were mediated by increased nervus vagus activity as reflected by high frequency heart rate variability (HF HRV).
Methods and results: HF HRV measurements and blood samples were taken from 180 patients at baseline and at 6 months. Spectral estimates of HF HRV were calculated from 5 minutes epochs of successive R-wave incidences of the ECG. Blood was analyzed for neopterin and interleukin 1 receptor antagonist (IL-1ra). The effect of the behavioral intervention on HF HRV was tested by an analyses of repeated measures. The association of changes in inflammation with changes in HF HRV was tested by an one-way analysis of variance. The behavioral intervention had a beneficial effect on HF HRV (repeated measures; F=4.99; p=0.03). HF HRV increased in those who displayed a decrease in IL-1ra and decreased in those who displayed an increase in IL-1ra (ANOVA; F=4.11; p=0.01). We found no association between changes in HF HRV and changes in neopterin concentrations.
Conclusions: The beneficial effect of the behavioral intervention on inflammatory markers is at least partly mediated by increases in nervus vagus activity as reflected by HF HRV.

Key words: *behavioral intervention, exhaustion, inflammation, high frequency heart rate variability*

Introduction

Inflammation is involved in all stages of atherosclerosis, from the initial lesion to the end-stage thrombotic complications [1]. The inflammatory status of atherosclerotic body compartments is relayed to the central nervous system (CNS) by humoral and neural routes. The humoral route dominates the immune-to-brain communication when circulating cytokine concentrations are high. When cytokine concentrations are low the CNS is still informed about the inflammatory status by the autonomic nervous system (ANS), in particular the afferent nervus vagus. Both routes can induce an anti-inflammatory response by activating the hypothalamic-pituitary-adrenocortical (HPA) axis to produce cortisol. High levels of cortisol suppress inflammation [2]. Afferent nervus vagus activity can further generate a rapid anti-inflammatory response that is partly mediated by the efferent nervus vagus. The stimulated efferent nervus vagus releases acetylcholine that deactivates macrophages by inhibiting the release of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and other cytokines (the “inflammatory reflex”) [3]. This suggests, as formulated by Tracey, that intentional modulation of nervus vagus activity may provide a therapeutic advantage for inflammatory disease. It is therefore of interest that autonomic functions (e.g. heart rate), which are normally under involuntary control, can be modulated by signals originating from higher brain centers. For instance, vagal control of heart rate (as reflected by the high-frequency (HF) component of heart rate variability (HRV)) can be enhanced through HRV biofeedback or paced breathing [4, 5].

A beneficial effect of a behavioral intervention on the inflammatory status of percutaneous coronary intervention (PCI) patients was described in part I of this article. The behavioral intervention reduced the odds of elevated concentrations of neopterin and IL-1 receptor antagonist (IL-1ra) at 18 months. Thus the question arose whether an increase in nervus vagus activity (i.e. an increase in HF HRV) may underlie this beneficial behavioral intervention effect on the inflammatory status of PCI patients. More specifically we tested the hypothesis that an increase in HF HRV during the behavioral intervention is associated with lowering of inflammation.

Methods

The selection of patients, the behavioral intervention and the immunological assessments were described in part I.

Participants

HRV was assessed in 268 consecutive patients. Of these patients, 19 patients were excluded because the assessment of HRV after the behavioral intervention was missing (e.g. patients refusal or technical problems in recording the electrocardiogram (ECG)). This meant that data of 249 patients were included in the statistical analyses. At baseline, of 180 patients both blood samples and HRV measurements were available. At 6 months, both blood samples and HRV measurements were available of 168 patients. Blood samples were collected (as described in part I) at baseline, at 6 months and at 18 months.

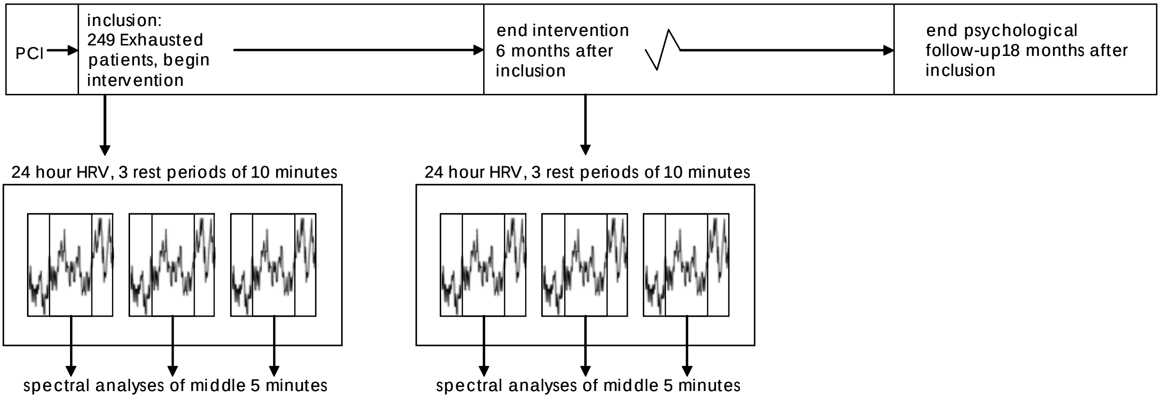
HRV measurement

Successive R-wave incidences of the ECG were detected with an accuracy of 1 ms and stored by an in-house portable device. The device monitored these R-wave incidences for at least 24 hours. The first 24-hour recording (baseline) was approximately two weeks after inclusion (i.e. 1.5 months after PCI) and the second 24-hour recording 6 months after baseline (i.e. 7.5 months after PCI). On the two occasions, patients arrived at the laboratory in the morning for the montage of ECG electrodes at the distal part of the sternum and at the sixth rib in the left axilla. After starting the device at approximately 11:00 AM, patients resumed their normal daily activities during the recording period. On both occasions, patients were requested to take three rest periods during their normal daily activities for about 10-15 minutes in a quiet sitting position, and to indicate the start and stop times of their rest periods in a logbook and on the device by pressing start and stop buttons. They were requested to take the first two rests during the first day with an inter-rest interval of at least four hours and to take the third rest during the second day early in the morning. In addition, they were requested to refrain from activities that could modify the placement of electrodes or harm the quality of the recordings (e.g. showering). Patients returned to the laboratory the next day, where they were disconnected from the device and the entire 24-hour recording of successive R-wave incidences was uploaded to a PC.

Detection of rest periods

Start and stop times of the rest periods were traced from the logbook of each patient and were used to locate the start and stop times in the 24-hour recording. The mean start times of the various rests at baseline were 2:05 PM (SD 5:04) for the first rest, 6:30 PM (6:43) for the second rest and 7:48 AM (3:44) for the third rest. At 6 months these times were 1:41 PM (5:29), 5:38 PM (7:31) and 7:09 AM (3:59) respectively. Each rest period, for which start and stop times corresponded to those of the logbook, were cut from the 24-hour recording and stored as a separate file of successive R-wave incidences. This resulted in a maximum of six files of about 10-15 minutes each per patient (i.e. three files during baseline and three at 6 months). To standardize for individual differences in the length of these rest periods, the middle five minutes of these files were cut and saved for further analysis (Figure 1).

Figure 1. Investigation protocol



ECG artifact detection and spectral analysis

Successive R-wave incidences were scrutinized for artifacts through visual inspection. Artifacts were corrected-omitting or inserting beats-by interpolation of the two preceding and two succeeding values [6]. Only recordings with less than 8 artifacts were corrected and included in the spectral analysis. Per patient and per rest period, the corrected 5-minute time series of R-wave incidences were subjected to a discrete Fourier transformation based on non-equidistant sampling of the R-wave incidences [7]. This allowed the calculation of the power spectra over a frequency range of 0.02-0.5 Hz, with a resolution of 0.01 Hz. For each 5-minute time series, the power distribution was calculated for the high frequency band (HF: 0.15-0.5 Hz), which is thought to reflect parasympathetic activity [8]. The resulting HF HRV power was averaged across the three rest periods as obtained per patient during baseline and at 6 months for further analyses.

Statistical analyses

The statistical analyses started by investigating whether drop out was associated with the study factors. Selective drop out may cause a restriction of range resulting in biased estimates of the association between changes in inflammation and changes in HF HRV. The Kruskall-Wallis (KW) test was used to test whether a potential loss of patients was associated to inflammatory markers and to HF HRV. There was a loss of patients (i.e. from 249 to 168 patients) when combining HF HRV data with immunological data. Patients with missing HF HRV data or blood samples at 6 months did not differ on HF HRV or inflammatory markers assessed at baseline. Patients with missing blood samples at 18 months did not differ on HF HRV assessed at baseline. Missing blood samples at 18 months were found to be associated with IL-1ra. Those with missing data had higher baseline concentrations of IL-1ra (KW; $\chi^2=3.47$; $p=0.06$). The selective dropout caused a restriction of range that biased the estimates of the association between vagal activity and inflammation, which possibly underestimated the association. Because of these missing values, analyses were restricted to baseline and 6 months measurements. Because HF HRV data were not normally distributed, log transformation of HF HRV data was employed.

Based upon the assumption that HF HRV was influenced by the behavioral intervention, the effect of the behavioral intervention on HF HRV was tested in 249 patients by a 2 (intervention vs control) by 2 (baseline and 6 months) analysis of repeated measures.

Immunological data were categorized into quartiles as described before [9]. To investigate whether changes in HF HRV during the behavioral intervention were associated with changes in inflammation, firstly, delta HF HRV was calculated (6 months-baseline). Secondly, three groups were formed based upon changes in a particular inflammatory marker between baseline and 6 months.

The first group included patients who displayed either stable low inflammation (i.e. concentrations in the lower three quartiles both at baseline and at 6 months) or stable high inflammation (i.e. concentrations in the highest quartile both at baseline and at 6 months). The second group included patients who displayed a decrease in inflammation (i.e. concentrations in the highest quartile at baseline and concentrations in the lower three quartiles at 6 months). The third group included patients who displayed an increase in inflammation (i.e. concentrations in the lower three quartiles at baseline and concentrations in the highest quartile at 6 months). To test for differences in delta HF HRV and changes in inflammation, an one-way ANOVA with post-hoc Bonferroni tests was used. Data processing and statistical analysis was performed using SPSS for Windows software, version 10.0 (SPSS, Chicago, Illinois). Significance levels were based on two-tailed tests, with α level set at .05.

Results

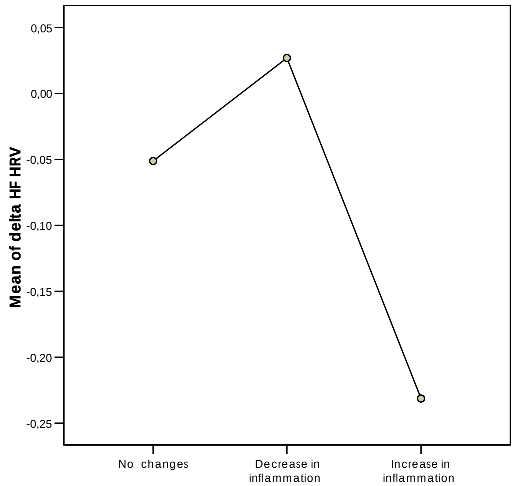
Effect of the behavioral intervention on HF HRV

The effect of the behavioral intervention on HF HRV was explored in 124 patients in the intervention group and 125 patients in the control group. Over the course of 6 months there was a beneficial effect of the behavioral intervention on HF HRV ($F=4.99$; $p=0.03$).

Association of HF HRV with IL-1ra

The mean delta HF HRV was significantly different between the three groups (group 1: n=141; group 2: n=17; group 3: n=19; ANOVA; $F=4.41$; $p=0.01$). The patients who displayed a decrease in inflammation (i.e. group 2) showed an increase in HF HRV, the patients who displayed an increase in inflammation (i.e. group 3) showed a decrease in HF HRV. Post hoc analyses showed that the overall effect was mainly due to the difference between groups 2 and 3 (Bonferroni; mean difference=0.26; $p=0.02$; Figure 2).

Figure 2. Changes in inflammation and HF HRV over 6 months



Changes in IL-1ra has three groups:
Group 1 (n=141) = patients with stable low or high inflammation, baseline and 6 months in either the lower three or upper quartile(s)
Group 2 (n=17) = patients decreased in inflammation, baseline in the highest quartile, after six months in the lower three quartiles
Group 3 (n=19) = patients increased in inflammation, baseline in the lower three quartiles, after 6 months in the highest quartile

Association of HF HRV with neopterin

There was no difference in mean delta HF HRV between the three groups (group 1: n=135; group 2: n=28; group 3: n=14; ANOVA; F=0.70; p=0.50). Patients who displayed an increase in inflammation (i.e. group 3) had the lowest HF HRV scores, however, post hoc testing was not significant.

Discussion

The main question addressed in this study was: is the beneficial effect of the behavioral intervention on inflammation mediated by the effect of the behavioral intervention on nervus vagus activity? The behavioral intervention had a beneficial effect on nervus vagus activity as reflected by HF HRV. We did not find an association for changes in neopterin with changes in HF HRV during the behavioral intervention. We observed an interaction between increases in HF HRV during the behavioral intervention in patients who decreased in IL-1ra and in patients who decreased in HF HRV and increased in IL-1ra.

These results at least partly support the proposed model of the inflammatory reflex by Tracey [3]. The nervus vagus influences inflammation through a fast and through a slow acting pathway. Peripheral ganglia of the nervus vagus have receptors for IL-1 and can inform the brain of low levels of inflammation. Upon stimulation of the nervus vagus by IL-1, acetylcholine is quickly released via the nervus vagus' descending pathway which has a local anti-inflammatory effect. Via a slow acting pathway a second anti-inflammatory effect is established. The stimulation of the nervus vagus by IL-1 informs and activates the HPA axis which leads to a production and systemic release of cortisol into the blood flow, a potent anti-inflammatory hormone.

We did not observe an association between changes in neopterin and changes in HF HRV. Thus the data do not support the hypothesis that the beneficial effect of the behavioral intervention on neopterin was mediated by an increase in nervus vagus activity. Neopterin is produced solely by activated macrophages under the influence of interferon (IFN) γ. IFN-γ, like IL-1 is one of the first cytokines to be expressed during the initial response to injury and critical in the development of atherosclerotic lesions [10]. IFN-γ is mainly inhibited by glucocorticoids [11], and thus by the slow acting pathway of the inflammatory reflex. It is therefore not unlikely that the beneficial effect of the behavioral intervention on neopterin was mediated by changes in cortisol. Unfortunately no assessments of cortisol could be made in this study. It is not unlikely that the behavioral intervention has a beneficial effect on neopterin through activating the HPA axis. However, we have no data that gives direct support to this contention.

The findings for IL-1ra are in line with the proposed model by Tracey. The behavioral intervention had a beneficial effect on high concentrations of IL-1ra (part I). In the current study we show that this effect is possibly a result of increased nervus vagus activity as measured by HF HRV. IL-1 is one of the first cytokines to be expressed during the initial response to injury [12]. By decreasing concentrations of IL-1, possibly one of the first steps in the atherosclerotic process can be slowed and it is therefore of possible clinical importance.

We cannot tell which part of the behavioral intervention is responsible for the increases in HF HRV, however, it is known that breathing relaxation therapy can increase HF HRV [13]. There is some evidence that relaxation intervention techniques may influence inflammatory cytokines and the inflammatory response [14]. The current study adds to the understanding of the effect of behavioral intervention techniques on inflammatory responses and the inflammatory reflex.

The statistical methods used in this sub-study are open for discussion. Some scientists prefer to use continuous scores instead of categorized scores, because statistics using continuous variables include all available information. Comparisons of categories may overestimate a relationship. When continuous scores of delta HF HRV and delta of the immune parameters were used, the association between changes in HF HRV and changes in immune parameters were not significant.
We used categorical data instead of continuous data because categorization of immunological variables is common in risk prediction [15, 16]. We did so in computing the risk of a new coronary event associated with inflammation [9], and in computing the effect of the behavioral intervention on inflammation, and followed this approach testing the hypothesis that the beneficial effect of the intervention on markers of inflammation is mediated by the effect of the behavioral intervention on nervus vagus activity. We observed that changes in HF HRV were associated with changes in IL-1ra, especially that those who experienced an increase of inflammation showed a decrease of HF HRV, and that those who had stable high or low values of inflammation did not change in HF HRV.

Although the major finding of this sub-study should be interpreted with caution because the major finding was not replicated when continuous scores were used instead of categories, it is of theoretical relevance. The EXIT study showed that a behavioral intervention on exhaustion reduces the risk of a late coronary event in those who did not suffer from an inflammatory disease and had no previous history of CAD [17]. The immunological sub-study showed that this effect is plausible from a biological perspective because it could be demonstrated that the intervention had a beneficial effect on high concentrations of IL-1ra. IL-1 is one of the first cytokines to be expressed during the initial response to injury, and is one of the first steps in the atherosclerotic process.

Thus, the EXIT study supports the contention that a behavioral intervention in coronary patients may reduce the risk of a new coronary event, because it has a demonstrable and plausible effect on inflammation.

Conclusion

The beneficial effect of the behavioral intervention on inflammatory markers is at least in part mediated by an increased nervus vagus activity as measured by HF HRV.

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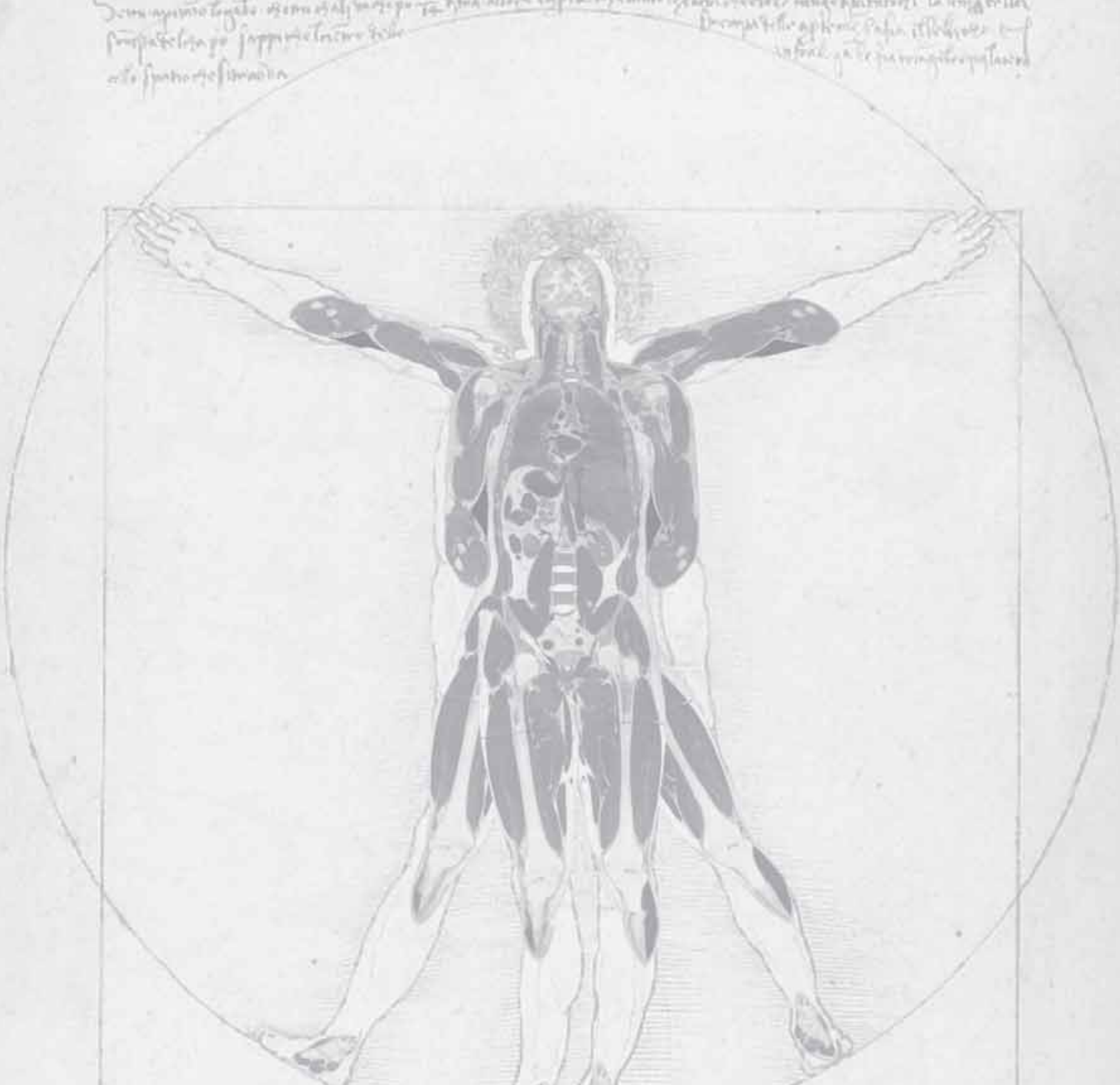
Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

Martijn Kwaijtaal



Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

Martijn Kwaijtaal

5

Exhaustion is associated with low macrophage migration inhibitory factor expression in patients with coronary artery disease

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Abstract

Objective. Macrophage migration inhibitory factor (MIF) is a protein secreted by immune cells and the pituitary gland, that may be associated with coronary artery disease (CAD) and the mental state of coronary patients. The first origin of MIF suggests positive, the second negative associations. The aim of the present study was to explore the direction of the association of MIF with CAD, and of MIF with exhaustion, if any.
Methods. The study comprised 194 patients, recently treated by percutaneous coronary intervention (PCI). All patients felt exhausted at the start of the study. Half of them entered a behavioral intervention program. MIF, C-reactive protein (CRP), interleukin (IL)-6, IL-1 receptor antagonist (IL-1ra) and neopterin were measured in blood collected six weeks after PCI (baseline), and 6 and 18 months after baseline. A single measurement of MIF was also available of 129 age- and sex-matched healthy individuals (reference group).
Results. At baseline MIF in PCI patients was significantly lower than in the reference group ($p<0.01$). New cardiac events occurred twice more often in the lowest quartile than in the highest quartile of MIF concentrations. However, the association was not significant ($\chi^2=2.27$; df=3; $p=0.52$). During follow-up MIF concentrations increased significantly in PCI patients ($p<0.001$). hsCRP, IL-1ra, IL-6 and neopterin concentrations did not change over this time period. Finally, at 18 months MIF concentrations were significantly lower in the exhausted patients than in the non-exhausted patients ($p=0.02$).
Conclusions. Together the data are suggestive of a negative association of MIF with CAD and of MIF with exhaustion. The observation that those patients who remained exhausted had lower concentrations of MIF fits into earlier observations that suggested that exhausted coronary patients may be characterized by a hypoactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis.

Key words: *angioplasty, inflammation, MIF, exhaustion, depression*

Introduction

Macrophage migration inhibitory factor (MIF) is a protein of potential interest to the field of psychosomatic medicine. MIF was first described as a T cell-derived cytokine that inhibits the random migration of macrophages [1]. MIF has since been shown to be expressed by other peripheral immune cells such as monocytes and macrophages [2]. As a pro-inflammatory cytokine, MIF exerts its action by blocking the inhibitory effects of glucocorticoids on the release of other pro-inflammatory cytokines (e.g. interleukin (IL)-1, IL-6, tumor-necrosis factor (TNF)-α). MIF is also expressed by endocrine organs involved in the stress response, especially by the pituitary gland [3]. Pituitary derived MIF has important roles in the periphery, such as antagonizing the effects of glucocorticoids [4]. Because MIF is produced by T-cells and by the pituitary it can be classified as a pro-inflammatory cytokine as well as a hormone.

Current knowledge regarding the association of MIF with coronary artery disease (CAD) is ambiguous. On the one hand, MIF induces expression of the intercellular adhesion molecule-1 by vascular endothelial cells, thus promoting atherosclerosis [5]. Inhibition of MIF results in a shift of neointimal atherosclerotic plaques towards a more stabilized plaque [6]. An upregulation of MIF during the progression of atherosclerosis towards inflammatory stages has been reported in humans [7]. Finally, MIF was found to have a positive but weak association with future CAD in the EPIC study [8]. These arguments suggest a positive association of MIF with CAD. On the other hand, high concentrations of MIF have been shown to promote neovascularization, especially during hypoxic stress, suggesting that MIF is cardioprotective [9-12].

To our knowledge the association of MIF with the mental state of coronary patients has not yet been investigated. We have approached the depressive symptomatology that may be observed in more than half of all coronary patients as a state of exhaustion caused by prolonged exposure to stress. Psychophysiological studies have found that exhausted subjects are characterized by higher levels of serological markers of inflammation (e.g. C-reactive protein (CRP), IL-6, TNF-α) [13, 14]. These observations suggest a positive association of MIF with exhaustion. Exhausted subjects are also characterized by lower levels of adenocorticotrope hormone (ACTH) and cortisol [15, 16]. This suggests that exhaustion is characterized by a decreased activity of the hypothalamic-pituitary-adrenocortical (HPA) axis. Hypoactivity of the HPA axis results in reduced inhibition of immune mediated inflammation [17, 18]. Because MIF is also a hormone secreted by the pituitary gland, these observations suggest a negative association of MIF with exhaustion.

The aim of the current study was to explore the direction of the association of MIF with CAD and of the direction of the association of MIF with a state of exhaustion, using blood samples of percutaneous coronary intervention (PCI) patients. To investigate whether MIF is a potential risk factor or a potential protective factor of CAD we posed the following questions:

1) Are MIF concentrations in the blood of PCI patients different from the MIF concentrations in a reference group? 2) What is the association of MIF concentrations with occurrence of new cardiac events in PCI patients? 3) Is the expression pattern of MIF different from the expression pattern of other pro-inflammatory cytokines during a period of 18 months following PCI?
To investigate the association of MIF with exhaustion we posed the following question: Are MIF concentrations in exhausted PCI patients different from MIF concentrations in non-exhausted PCI patients?

Methods

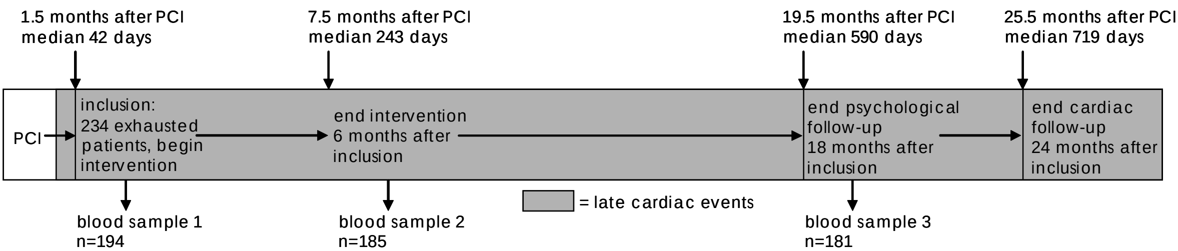
Patients

The present study is part of the Exhaustion Intervention Trial (EXIT), a multi-center randomized controlled trial designed to test whether lowering of exhaustion by a behavioral intervention reduces the risk of new cardiac events in PCI patients [19]. Group discussions, breathing-relaxation therapy, hostility therapy and educational sessions were applied as behavior modification techniques to reduce exhaustion, and to support recovery by making rest more efficient. EXIT consisted of 10 weekly sessions with groups of six patients, followed by four monthly sessions, and required that patients fulfilled strict criteria for exhaustion at entrance. In short, exhaustion was assessed in two stages. First, the Maastricht Questionnaire (MQ) was used (23 items; range 0-46) to establish whether patients met a MQ cut-off score of $\geq$14 to enter the second stage. In this stage, the Maastricht Interview for Vital Exhaustion (MIVE) was administered to establish whether patients also met a MIVE cut-off score of $\geq$7 to be included in EXIT. The MIVE better predicts future cardiac events than the MQ and consists of 23 questions (range 0-23) [20]. Further details of inclusion and exclusion criteria, cut-off values and applied behavioral intervention techniques, are presented elsewhere [19].

Collection of blood samples

In one participating center (Maastricht), blood samples were collected on three occasions (Figure 1).

Figure 1. Flow chart of patient selection and assessments



All three blood samples were collected of 172 patients

A medical specialist screened the medical records of the PCI patients for the use of immunosuppressive medication. This resulted in the exclusion of 18 patients. Therefore, the first blood sample (baseline) was available of 194 patients. The median interval between PCI and baseline was 42 days.

The second blood sample was obtained 6 months after baseline and available from 185 patients (i.e. data of 9 patients are missing due to either laboratory processing errors or because patients did not show up, refused to give blood or had died). The last blood sample was obtained 18 months after baseline and available of 181 patients (i.e. data of 13 patients are missing due to loss-to-follow-up). In sum, all 3 blood samples were available of 172 of the initial 194 patients.

To compare MIF concentrations of PCI patients with values in a reference group, a single blood sample was also available of 129 age- and sex-matched individuals who denied having any cardiovascular symptoms and who were apparently healthy. This group was not tested for exhaustion. Of all blood samples, 90% was collected in the morning (range 8:30AM-12:00PM). The institutional review board of the participating center approved the study protocol, and all participants gave written informed consent.

Measurement of MIF

Plasma samples were collected from ethylenediaminetetraacetic-treated (EDTA) blood and stored at -20°C until further processing. An enzyme-linked immunosorbent assay (ELISA) for human MIF has been developed using the 4-span approach earlier described by Grebenschikov et al. [21]. Antibodies were raised in chicken and rabbits using rhMIF as immunogen. The sandwich structure employed includes four different antibodies (Abs), viz. a coating Ab (duck anti-chicken), a capture Ab (chicken anti-hMIF), a trapping Ab (rabbit anti-hMIF) and finally a detection Ab (HRP-labeled goat anti-body (2h at 37ºC). The incubation with the unknowns, reference samples and the standards took place overnight at 4ºC (approximately 16h). The incubation with trapping antibody as well as the subsequent incubation with detection antibody was performed for 2h at ambient temperature. The incubation with substrate solution was performed in darkness for 30 min at ambient temperature. Color reaction was stopped by the addition of H_2SO_4 and the optical density was measured at 492 nm within 30 min. In each run, a reference preparation was run to check inter-assay variability and to monitor overall performance [22]. The analytical sensitivity of the assay is 39 pg/ml. The precision profile showed a coefficient of variation (CV) of 20% at 45 pg/ml (i.e. functional sensitivity) decreasing to 7% at higher levels. For estimation of the accuracy of the method a lyophilized reference preparation (marked 140799) is used. The mean hMIF concentration in 140799 was 20.7 ng/mL, the intra-assay and the inter-assay CV amounted to 6.0% (n=8) and 12.0% (n=11, over a period of 13 months), respectively.

Measurement of other pro-inflammatory markers

Blood was allowed to clot at room temperature and centrifuged. Serum samples were stored at -20°C until further processing. hsCRP, IL-1ra and IL-6 were measured in serum using quantitative ELISAs (DiaMed Eurogen, Turnhout, Belgium). Serum was diluted 1000x for hsCRP, whereas IL-1ra and IL-6 were quantified in undiluted serum. Test samples and standard solutions were incubated on pre-coated ELISA plates. A biotinylated secondary antibody against the relevant inflammatory marker was used. Peroxydase conjugated streptavidin was then applied to bind to the biotinylated antibody and after washing, a tetramethylbenzidine-solution (TMB) was used to stain the remainder of the bound streptavidin; the reaction was stopped by adding sulfuric acid. Optical densities were read using a PowerWaveX Reader (MWG Biotech, Ebersberg, Germany) at 450 nm, data were calculated using KC4 software (MWG Biotech, Ebersberg, Germany). Neopterin was measured using a competitive ELISA (IBL-Hamburg, Hamburg, Germany) in accordance with the manufacturer's recommendations. All serum samples were analyzed in duplicate. Sensitivity of all tests was calculated by the mean of 6 zero-values +3 standard deviations extrapolated on the standard curve. Intra-assay and inter-assay CV for hsCRP was 5.1% (n=10), and 14.3% (n=7), respectively. Intra-assay en inter-assay CV for IL-1ra, IL-6 and neopterin were 6.1% (n=10) and 9.2% (n=7), 5.5% (n=10) and 6.8% (n=7), and 3.6% (n=11) and 7.6% (n=6), respectively.

Assessment of new cardiac events

To investigate whether MIF is associated with new cardiac events in PCI patients, a research assistant and a cardiologist (fifth author) assessed the occurrence of new cardiac events (defined as re-PCI, coronary artery bypass graft surgery (CABG), MI or cardiac death) through inspection of the medical records. Hospitalizations for unstable angina were not counted as new cardiac events. The mean cardiac follow-up was 24 months. To ensure that no deaths were missed, the family physicians received a letter asking whether a patient was still alive and, if the patient had died, what had been the cause of death. A 100% cardiac follow-up and cause of death was achieved, so no loss-to-follow-up or loss of events occurred. Cardiac events were defined as events occurring at least one month after PCI (Figure 1). Cardiac events occurring within one month after PCI were defined as early events and excluded from the present analyses, because a number of these events were missed due to the design of the study. Furthermore, early cardiac events are not a reflection of the progression of atherosclerosis, but are usually the result of inadequate intervention, recoil of the vessel wall or neointimal hyperplasia (an effect of the vascular damage due to PCI) [23].

Macrophage migration inhibitory factor and exhaustion

The question whether MIF is associated with exhaustion could not be studied with data obtained at the start of EXIT, because only exhausted PCI patients were included in EXIT. The MIVE was re-administered 18 months after inclusion and allowed us to investigate whether exhausted and non-exhausted PCI patients displayed similar MIF concentrations. Many scientists approach the symptoms of fatigue, loss of energy and increased irritability as a clinical depression. Therefore, we also analyzed the association of MIF with major depression as assessed at baseline and 18 months by the SCID.

Statistical Analysis

Non-parametric testing was used as concentrations of MIF and inflammatory markers were not normally distributed (evidenced by Kolmogorov-Smirnov goodness of fit testing) even after log-transformation. To test whether PCI patients have MIF concentrations that are different from those of the reference group, the Mann Whitney U test was applied. To test whether MIF concentrations are associated with occurrence of new cardiac events; Mann Whitney U test was applied to test for differences between baseline MIF concentrations in the group with late cardiac events versus the group without late cardiac events. In addition, baseline MIF concentrations were categorized into quartiles. Categorization of skewed distributions of inflammatory markers is common in risk prediction [24, 25]. A crosstab analysis with χ^2 significance testing was applied to explore the direction of the association of MIF with new cardiac events, if any. In addition, a χ^2 test for trend was performed because it is more informative with respect to whether the risk increases from lower to higher quartiles. To test whether MIF increased or decreased during follow-up the median concentrations observed at baseline, 6 months and 18 months were compared using the Friedman rank test for related samples. The same procedure was used to investigate the changes in the expression of other inflammatory markers.

At 18 months the MIVE was re-administered. To establish whether PCI patients were exhausted or not at that time, the same cut-off was applied (i.e. MIVE $\geq$7) that was used to include patients in EXIT. Mann Whitney U test was applied to test whether the MIF concentrations in the exhausted group were different from those in the non-exhausted group. The association of MIF with exhaustion at 18 months was also calculated using bivariate correlations of the continuous variables (Spearman's correlation). We also investigated whether the intervention influenced MIF concentrations, using MANOVA. In this analysis MIF data obtained at baseline, at 6 months and at 18 months were included. Data processing and statistical analyses were performed using SPSS for Windows software, version 11.0 (SPSS, Chicago, Illinois). Significance levels were based on two-tailed tests, with α level < 0.05.

Results

MIF concentrations in PCI patients and in a healthy reference group

MIF concentrations in blood were lower in the 194 PCI patients at baseline than in the reference group (PCI patients: median 39.6 ng/ml; 95% range 6.8-352.8 ng/ml vs reference group: median 67.5 ng/ml; 95% range 20.8-171.1 ng/ml; Mann Whitney U Z=-3.8; p<0.01). After 18 months of follow-up, median MIF concentrations (median 69.9 ng/ml; 95% range 19.9-386.4 ng/ml) in the PCI patients were slightly higher than the concentrations of the reference group (Mann Whitney U Z=-1.65; p=0.10).

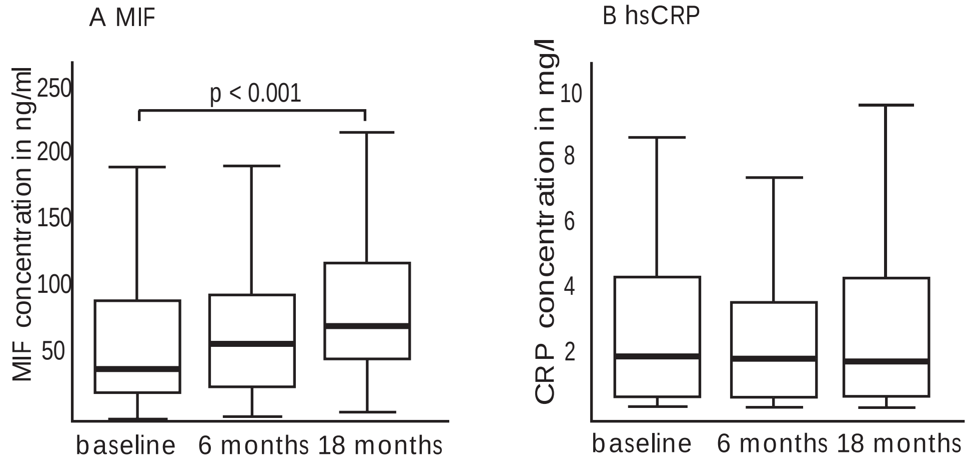
MIF concentrations and occurrence of new cardiac events in PCI patients

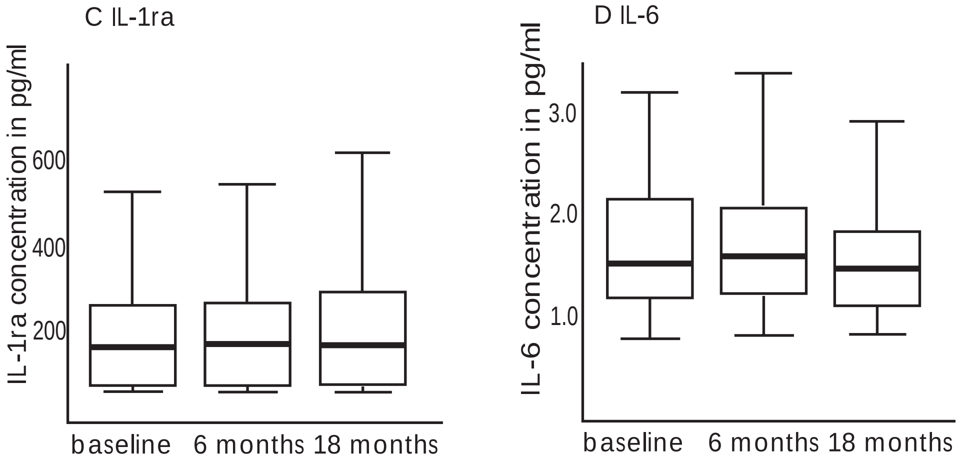
Of the 194 patients, 2 (1%) suffered from an early cardiac event and were excluded from the analysis. Of the remaining 192 patients, 31 (16%) suffered from a late cardiac event. The "late cardiac event" group did not differ significantly from the "no event" group with respect to demographic (e.g. age, gender, smoking) and medical characteristics (e.g. blood pressure, diabetes, major depression), except that ACE inhibitor intake was more frequent in the "late cardiac event" group (χ^2=7.1; p<0.01). Mann Whitney U showed that the MIF concentrations of the late cardiac event group was lower than those in the group without late cardiac events, although this association was not significant (Mann Whitney U Z=-1.08; p=0.28). To further investigate the association, MIF concentrations were categorized into quartiles (Q1 to Q4). MIF concentrations in Q1 ranged from 2.51-22.97 ng/ml, and 10 (21%) patients in this subgroup suffered from a late cardiac event. In Q2 (MIF: 22.98-39.59 ng/ml), 7 (14%) patients suffered from a late cardiac event. In Q3 (39.60-90.29 ng/ml) and Q4 (90.30-618.08 ng/ml), late cardiac events were observed in 9 (18%) patients and 5 (10%) patients, respectively (χ^2=2.27; df=3; p=0.52). Thus, a trend towards a decrease in cardiac events over the quartiles could be observed in the data. However, the χ^2-test for trend did not show a significant trend for new cardiac events (χ^2=1.32; df=1; p=0.25). However, this trend was not significant. To control for the possible influence of ACE inhibitors the data were also analyzed by Cox regression analysis, controlling for ACE inhibitors. Results showed that the negative association could not be attributed to the use of ACE inhibitors (data not shown).

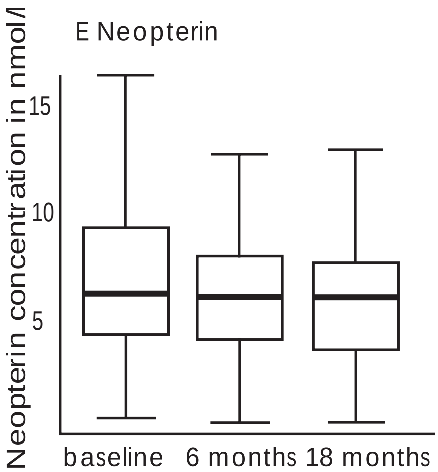
Expression pattern of MIF and other pro-inflammatory markers

The expression patterns of MIF, hsCRP, IL-1ra, IL-6 and neopterin over the 18 months of follow-up are shown in Figure 2.

Figure 2. hsCRP, IL-1ra, IL-6, neopterin and MIF concentrations in blood over 18 months of follow-up after PCI







Boxes represent 50% of all samples and thick line in boxes is the median concentration. Bars represent 95% CI, non-parametric Friedman Rank test was used. N for all groups was 172.

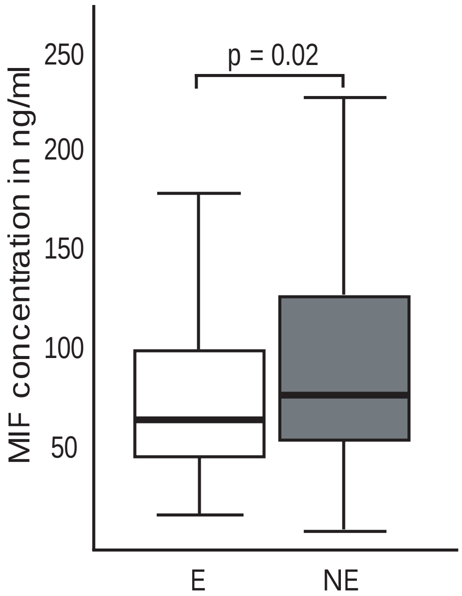
The MIF concentration (figure 2A) increased significantly over 18 months of follow-up (baseline: median 39.9 ng/ml; 95% range 6.7-347.6; 6 months: median 53.4 ng/ml; 95% range 4.9-321.3; 18 months: median 69.6; 95% range 19.9-386.4; Friedman rank χ^2=18,7; p<0.001). There were no significant increases or decreases in the expression patterns of hsCRP, IL-1ra, IL-6 and neopterin.

MIF concentrations in exhausted and non-exhausted PCI patients

At 18 months 92 patients (51%) still felt exhausted, (MIVE: mean 12.3; SD ± 4.3), whereas 89 patients (49%) were no longer exhausted (MIVE: mean 2.7; SD ± 1.9). The median MIF concentration in the exhausted PCI group (63.4 ng/ml; 95% range 19.9-440.5 ng/ml) was significantly lower than the median MIF concentration in the non-exhausted group (79.1 ng/ml; 95% range 17.5-386.2 ng/ml; Mann Whitney U Z=-2.3; p=0.02; Figure 3).

Those who still fulfilled the inclusion criterion for exhaustion at 18 months were characterized by lower concentrations of MIF. However, when bivariate correlations were used tot test the association between MIF and exhaustion there was no significant association (*r*=-0.04; p=0.56).

Figure 3. MIF concentration in blood after 18 months in exhausted and non-exhausted patients



Total patient population is divided in exhausted versus non-exhausted based upon 18 months exhaustion scores. White box represents the exhausted group, grey box represents the non-exhausted group. Bars represent 95% CI, boxes represent the 50% range of the variable and the thick line in the boxes is the median. Mann Whitney U test was performed to test for differences between the two groups. N for the exhausted group was 92, N for the non-exhausted group was 89.

At baseline 30 (16%) patients suffered from a major depression (MD). MD was not associated with MIF (Mann Whitney U Z=-0.73; p=0.46). At 18 months 11 (6%) patients suffered from a MD. The median concentration of MIF in the depressed group (50.4 ng/ml; 95% range 30.45-65.40 ng/ml) was significantly lower than the median concentration of the non-depressed group (74.7; 95% range 19.9-429.4 ng/ml; Mann Whitney U Z=-2.51; p=0.01).

The effect of the intervention on MIF

There was no effect of the behavioral intervention on the concentrations of MIF (MANOVA: F=0.12; df=3; p=0.95).

Discussion

MIF plasma concentrations in patients approximately 6 weeks after PCI were significantly lower than those of the healthy reference group. MIF plasma concentrations increased significantly over 18 months of follow-up in these PCI patients and returned to the concentrations observed in the reference group. The pattern of MIF expression differed from the pro-inflammatory markers CRP, IL-1ra, IL-6 and neopterin (which were stable) in these PCI patients. MIF concentrations were not associated with late cardiac events in PCI patients, although, a negative trend was observed for late cardiac events versus MIF concentrations. At 18 months of follow-up the plasma concentrations in the non-exhausted group were significantly higher than in the exhausted group.

MIF is a pro-inflammatory cytokine, which can either directly or indirectly promote the production of a large number of other pro-inflammatory molecules, including cytokines [26]. Because MIF is a marker of inflammation and because CAD is an inflammatory disease one may expect that the median MIF value observed in PCI patients at baseline would be higher than the median value of

a healthy reference group. We observed the opposite. There was a trend towards lower occurrence of late cardiac events in those with higher concentrations of MIF. However, this association was not significant. This may be due to the limited power of the current study. Together, our data suggest that PCI patients are characterized by relatively low concentrations of MIF that tend to increase in an 18-month follow-up.
Those who remained exhausted had significantly lower concentrations of MIF compared to those who were no longer exhausted at 18 months. These results suggest that MIF and exhaustion were negatively associated. Lower MIF concentrations were also observed among those who were depressed at 18 months. MIF is produced by a variety of sources such as immune cells and organs throughout the body, including the anterior part of the pituitary gland [26]. It has been reported that in exhausted subjects the activity of the HPA axis is decreased [16, 27]. These observations fit into earlier observations which suggested that a decreased activity of the HPA axis may underlie the mental state of coronary patients who feel exhausted or depressed [16, 27]. No data on ACTH and cortisol were collected in the current study. However, Beishuizen et al. observed that MIF in plasma is persistently elevated in septic patients, in parallel to plasma cortisol concentrations [28]. This gives some additional support to the suggestion of a decreased HPA activity in exhausted-depressed patients. However, MIF and exhaustion were not correlated when exhaustion was assessed as a continuous variable. Furthermore, major depression at baseline was not related to MIF concentrations at baseline. Therefore, the data do not prove that low MIF concentrations are mainly observed in the patients who are exhausted or depressed. Further explorations of changes in MIF concentrations in those who stayed depressed or became depressed during the follow-up period failed because of the small numbers.

It is not likely that these results were influenced by immunosuppressive medication because all patients who used this type of medication (e.g. glucocorticoids, rheumatoid arthritis medicine, i.e. medications produced specifically to reduce inflammation) were excluded from the analyses. Other medications with possible anti-inflammatory side-effects (i.e. lipid-lowering drugs or statins) were used by 83% of all PCI patients, however these medication were also used at 18 months of follow-up and did therefore not explain an increase in MIF concentrations over the follow-up period. It is also unlikely that the results presented above were influenced by the behavioral intervention offered to half of the patients, because the intervention had no effect on MIF at all. The use of MANOVA is debatable because of the skewed distribution of the data, therefore we also made pairwise comparisons of changes in MIF in the intervention and the control group using non parametric tests. These analyses confirmed the negative conclusion resulting from the MANOVA analysis.

Several studies indicate that MIF-neutralization might be a new target to combat the progression of atherosclerosis [29, 30]. Inhibition of MIF in vascular injured Apo-E-deficient mice resulted in stabilization of atherosclerotic plaques, which might be attributable to a reduction of monocyte recruitment mediated by endothelial MIF [6]. Neutralization of MIF is reported to stop the pro-inflammatory effects of MIF and therewith the progression of atherosclerosis [30]. However, when MIF is neutralized, no biologically active MIF is present. One of the most important actions of MIF is the host's immunological defence against anomalies (e.g. bacterial infection, damage to vessel wall) [31]. Neutralization of MIF will take this function away, furthermore, the activity of MIF in collateral circulation will also be blocked [9]. There are several studies investigating the angiogenic properties of MIF supporting a positive effect for MIF in angiogenesis [10, 32]. One study supports a role for MIF as a therapeutic inducer of neovascularization in the development of collateral circulation in CAD [9]. Therefore, trials neutralizing MIF in CAD patients will have to be considered with extreme caution.

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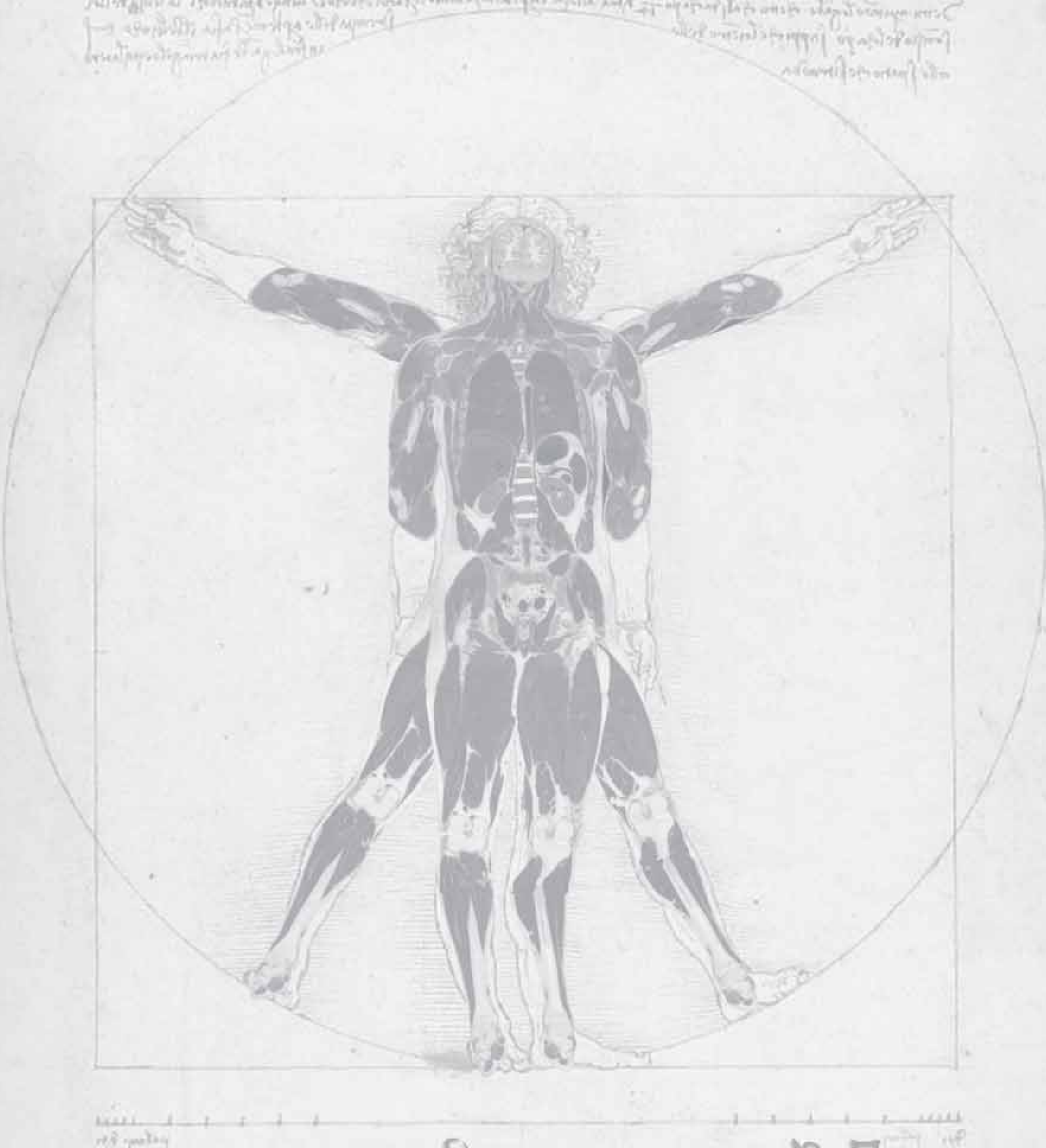

Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

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6

Impact of pathogen burden, C-reactive protein and neopterin on cardiac events: it's the combination that counts

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Submitted

Abstract

Pathogen burden (PB i.e. the aggregate number of pathogens to which an individual has been exposed during life) and inflammation have been suggested to play a role in coronary artery disease (CAD). We hypothesized that a combined index of PB and C-reactive protein (CRP) increases the risk of late cardiac events in percutaneous coronary intervention (PCI) patients. Secondly, we hypothesized that a combined index of PB and neopterin, a marker for monocyte/macrophage activation, increases the risks. Patients (196) recently treated by successful PCI were studied. Serum samples collected approximately 6 weeks after PCI were analyzed for immunoglobulin-G antibodies to 4 common herpes viruses and *Chlamydia pneumoniae*. In addition, CRP and neopterin were measured. In the mean cardiac follow-up of 24 months, 15 late cardiac events occurred. Multivariate analyses were used to determine the prognostic value. PB, CRP and neopterin did not increase the risk of late cardiac events. The risk of a late cardiac event was increased when combining high values for PB and CRP (RR=4.2) or high values for PB and neopterin (RR=3.8). This supports the contention of Zhu that "infections are not innocent bystanders but through inflammatory responses they provoke play a causal role in genesis of atherosclerosis".

Key words: *pathogen burden, inflammation, PCI*

Introduction

Atherosclerotic plaques often harbor multiple pathogens, including Herpes Simplex virus (HSV), Varicella Zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV) and *Chlamydia Pneumoniae* (CPn) [1, 2]. These pathogens may promote atherogenesis by infecting the arterial wall, altering vascular cell lipid metabolism, induction of cytokines and growth factors, and by procoagulant effects on the vascular endothelium [3]. Notwithstanding this evidence, an increased risk of CAD has not consistently been found for each of these individual pathogens [4, 5]. Recent studies, therefore, have focused on CAD risk and the aggregate number of pathogens to which individuals have been exposed during their life (i.e. pathogen burden or PB) [6-9]. Although negative results have been reported [10, 11], most studies showed that an increased risk of CAD is directly associated with high PB. Zhu and others, however, showed that the risk of cardiac morbidity and mortality is increased when evidence of prior pathogen infection (seropositivity) is coupled with increased concentration of C-reactive protein (CRP) [5, 12-14], thus suggesting that PB is not directly associated with cardiac risk, but through an interaction of PB with inflammation. Using data of a follow-up study in percutaneous coronary intervention (PCI) patients, both proposals were investigated by testing two hypotheses: 1) is a combined index of PB and CRP, a better predictor of late cardiac events than PB and CRP by themselves? 2) is a combined index of PB and neopterin concentrations, a better predictor of late cardiac events than PB and neopterin by themselves? The distinction between these two inflammatory markers was made because CRP reflects systemic inflammation [15], whereas neopterin, is a specific marker for monocyte/macrophage activation [16]. Activated monocytes play an important role in the pathogenesis of atherosclerosis.

Confirmation of the hypothesis that the combined index of elevated PB and elevated markers of inflammation increases the risk of CAD would give additional support to the contention of Zhu et al that pathogens are not innocent bystanders, but combined with the presence of inflammation play a causal role in the genesis of atherosclerosis.

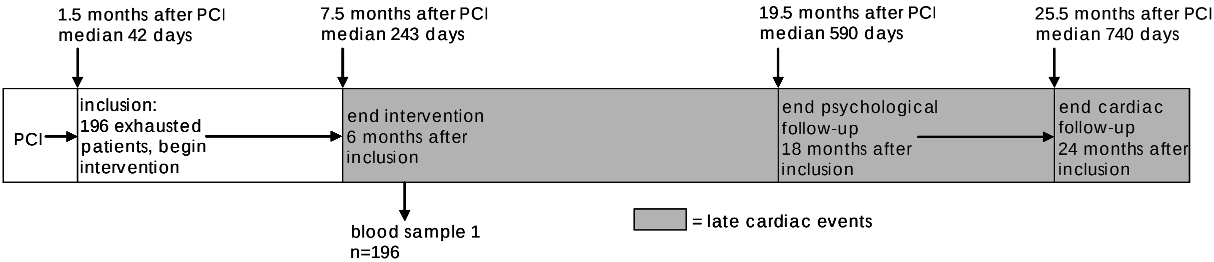
Methods

Participants

The present study is part of the Exhaustion Intervention Trial (EXIT), a multicenter randomized controlled trial designed to test the hypothesis that lowering of exhaustion by a behavioral intervention reduces the risk of a new cardiac event in PCI patients. The design of the study, inclusion and exclusion criteria, and the assessment of new cardiac events have been described elsewhere [17]. Briefly, participants of EXIT were 710 patients, aged 35-68 years, who felt exhausted after successful angioplasty (i.e. a reduction of 50% or more of the culprit lesion), without early complications. Exhaustion was measured using the Maastricht Questionnaire (MQ) [18] and in the second phase, the Maastricht Interview for Vital Exhaustion (MIVE) was used [19]. Only patients who fulfilled strict criteria for exhaustion were included in the study. The median interval between PCI and inclusion in EXIT was 27 days.

For the present prospective sub-study blood samples were taken from 196 patients in one of the participating centers (Maastricht). Blood collection was approximately two weeks after inclusion (i.e. median 42 days after PCI). The occurrence of new cardiac events (defined as re-PCI, coronary artery bypass graft surgery (CABG), myocardial infarction (MI) or cardiac death) was assessed through inspection of the medical records. The median cardiac follow-up time was 24 months. To ensure that no deaths were missed, the family physicians received a letter asking whether a patient was still alive and, if the patient had died, what had been the cause of death. A 100% cardiac follow-up and cause of death was achieved. Late cardiac events were defined as new cardiac events occurring 6 months after inclusion in the study. Cardiac events occurring within 6 months after inclusion were excluded from the present analyses, because a number of events occurring within 6 weeks after PCI were missed due to the design of the study. Furthermore, early cardiac events are in general not a reflection of the progression of atherosclerosis, but are classified as the result of an inadequate intervention, recoil of the vessel wall or neointimal hyperplasia (an effect of the vascular damage due to PCI) [20]. Figure 1 shows a flow chart of the current sub-study.

Figure 1. Flow chart of patient selection and assessments



The institutional review board of the participating center approved the study protocol, and all participants gave written informed consent.

Serological Techniques

Blood was allowed to clot at room temperature and centrifuged. Serum samples were stored at -20°C in anticipation of further processing. The serum samples were tested for immunoglobulin G (IgG) antibodies to HSV, VZV, EBV, CMV, and CPn. Antibodies to HSV, EBV, VZV and CPn were quantified using enzyme-linked immunosorbent assays (ELISA). ELISA-kits for HSV and EBV were purchased from Virion \ Serion (Würzburg, Germany), ELISA-kits for VZV were purchased from Euroimmun (Lübeck, Germany) and ELISA-kits for CPn were purchased from AniLabsystems (Helsinki, Finland). Manufacturer's instructions and guidelines were used for quantification. Seropositivity to HSV is defined as an IgG value ≥30 AU/ml; EBV ≥20 AU/ml; VZV ≥100 AU/ml and CPn ≥45 AU/ml.

Antibodies to CMV were quantified with the Microparticle Enzyme Immunoassay (Abbott Laboratories, Hoofddorp, Netherlands), using the Axsym automated analyzer. Seropositivity to CMV is defined as an IgG value ≥15 AU/ml. When samples reached the maximum test value, samples were diluted 10x and retested.

Immunological techniques

The markers of inflammation (high sensitive CRP and neopterin) were measured in serum using quantitative ELISA. ELISA-kits for high sensitive CRP were purchased from DiaMed Eurogen (Turnhout, Belgium). Serum for high sensitive CRP was diluted (1000x). Test samples and standard solutions were incubated on pre-coated ELISA plates. A biotinylated secondary antibody against the relevant inflammatory marker was used. Peroxydase conjugated streptavidin was then applied to bind to the biotinylated antibody and after washing, a tetramethylbenzidine-solution (TMB) was used to stain the remainder of the bound streptavidin; the reaction was stopped by adding sulfuric acid. Optical densities were read using a PowerWaveX Reader (MWG Biotech, Ebersberg, Germany), data were calculated using KC4 software (MWG Biotech, Ebersberg, Germany). Neopterin was measured using a competitive ELISA (IBL-Hamburg, Hamburg, Germany) in accordance with the manufacturer's recommendations. All serum samples were analyzed in duplicate. Sensitivity of the tests was calculated by the mean of 6 zero-values +3 standard deviations extrapolated on the standard curve. All samples that were below the sensitivity value were discarded and replaced with the value of sensitivity of the corresponding assay. Intra-assay and inter-assay coefficient of variation (CV) was for hsCRP 5.1% (n=10) and 14.3% (n=7), respectively, and for neopterin: 3.6% (n=11) and 7.6% (n=6), respectively.

Statistical analysis

To explore demographic and medical characteristics that may confound the association between serology, inflammation and the occurrence of cardiac events, ANOVA and χ^2 were used to compare groups with and without cardiac events. A PB index was created by the aggregate number of seropositive tests to IgG antibodies for HSV, EBV, CMV, VZV and CPn. High PB was defined as seropositivity to all five pathogens. Neopterin concentrations were categorized in quartiles [21]. For CRP a cut-off value of 3.0 mg/l was applied as recommended by the Centers for Disease Control and Prevention and the American Heart Association [22]. Top quartile was coded as 1 and the lowest three quartiles were coded as 0. To test the direct association of PB, CRP and neopterin with late cardiac events, Cox proportional hazards analyses was used, controlling for age and gender. To test the interaction of PB and inflammatory markers, exposed and non-exposed groups were constructed. An exposed group was defined as patients with a high PB and high concentration of either CRP or neopterin. A non-exposed group was defined as 1) patients with a low PB and any concentration of either CRP or neopterin, or 2) patients with a high PB and low concentrations of either CRP or neopterin. The interaction of PB and inflammation was assessed by calculating the relative risks (RR) of late cardiac events associated with these combined indices by Cox proportional hazards analyses, controlling for age, gender. We also tested whether the predictive power of these combined indices were mainly due to the PB or to the inflammatory components by calculating standardized coefficients. Standardization of the regression coefficients allows a direct comparison of the strength of the associations.

Data processing and statistical analysis was performed using SPSS for Windows software, version 10.0 (SPSS, Chicago, Illinois). Significance levels were based on two-tailed tests, with α level set at .05.

Results

Demographic and medical characteristics of the study population

During the cardiac follow-up period, an early cardiac event occurred in 18 of 196 patients (9%), a late cardiac event occurred in 15 of 196 (8%) patients, and 163 of 196 patients (83%) had no cardiac event. Of the in total 15 late cardiac events 10 patients underwent re-PCI and 5 patients underwent CABG. Furthermore, of these 15 patients 12 patients suffered from 1 cardiac event, 2 patients from 2 cardiac events and one patient suffered from 3 cardiac events after index PCI. Demographic and medical characteristics observed at baseline in the group with cardiac events and no cardiac events are presented in Table 1. No significant differences were found between these groups in demographic and medical characteristics.

Table 1 Demographic and medical characteristics of PCI patients with late cardiac events and PCI patients without cardiac events

	Late event (n=15)	No event (n=163)	p
Demographics			
Age	56.1 ± 6.3	53.7 ± 7.2	0.25
BMI	27.4 ± 3.7	27.2 ± 4.1	0.79
Blood pressure			
Systolic	138.9 ± 19.3	131.4 ± 19.8	0.16
Diastolic	88.3 ± 12.9	83.8 ± 10.0	0.10
Gender			
Male	13 (87%)	131 (80%)	0.45
Female	2 (13%)	32 (20%)	
Behavioral intervention group			
Intervention	7 (47%)	79 (49%)	0.89
Control	8 (53%)	84 (51%)	
Smoking			
Current	1 (7%)	27 (17%)	0.54
Stopped	13 (86%)	121 (74%)	
Never	1 (7%)	15 (9%)	
Medical characteristics			
Diabetes	1 (7%)	19 (12%)	0.56
Chronic painful condition	3 (20%)	20 (12%)	0.3
Cardiac history			
Previous MI	4 (27%)	44 (27%)	0.95
Previous CABG	2 (13%)	15 (9%)	0.62
Previous PCI	3 (20%)	23 (14%)	0.56
Indication for PCI*			
Stable Angina	0	14 (9%)	0.90
Unstable Angina	10 (66%)	95 (58%)	
Myocardial Infarction	4 (27%)	37 (23%)	
Post MI angina	1 (7%)	17 (10%)	
Stenoses after PCI			
0	7 (47%)	76 (47%)	0.13
1	1 (6%)	47 (29%)	
2 or more	7 (47%)	40 (24%)	
Stent implanted	13 (87%)	110 (68%)	0.13
Medication approximately 1.5 month (median 42 days) after PCI			
Ace inhibitor	5 (33%)	31 (19%)	0.32
Diuretics	2 (13%)	20 (12%)	0.94
Beta-blocker	11 (73%)	121 (74%)	0.84
Calcium antagonists	8 (53%)	56 (34%)	0.51
Statins	14 (93%)	129 (79%)	0.20
Nitrates	13 (87%)	129 (79%)	0.54
PB and inflammatory markers			
High PB	6 (46%)	56 (34%)	0.37
High CRP	7 (54%)	51 (31%)	0.09
High Neopterin	5 (39%)	40 (24%)	0.26

Fisher's exact test

Risk of a cardiac event associated with PB and inflammatory markers

A high PB was observed in 66 (34%) of the patients. An elevated level of CRP was observed in 58 (30%) of the patients, and an elevated level of neopterin in 45 (23%) of the patients. A high PB did not increase the risk of late cardiac events (RR=1.6; 95%CI .60-4.54; p=0.34). High baseline concentrations of CRP (RR 2.4, 95%CI .80-7.33, p=0.12) did not raise the risk of a late cardiac event. In addition, high concentrations of neopterin did not raise the risk of late cardiac events (RR 1.7, 95%CI .56-5.46, p=0.34).

Risk of a cardiac event associated with simultaneous presence of high PB and inflammation

Twenty patients were identified with a high PB and high concentrations of CRP, and 14 patients with a high PB and high concentrations of neopterin. No associations were observed between PB and CRP (χ^2=0.01;p=0.95) and between PB and neopterin (χ^2=0.37; p=0.55). The simultaneous presence of high levels of PB and CRP increased the risk of a late cardiac event significantly (RR=4.16;95%CI 1.20-14.39;p=0.02). The simultaneous presence of a high level of PB and neopterin also increased PB the risk of a late cardiac event (RR=3.76;95%CI 1.01-13.99;p=0.05). Thus, PB increases the risk of CAD when coupled with inflammation, controlled for age and gender (Table 2). The inclusion of "intervention-no intervention" in the Cox proportional hazard analysis to control for the effect of the behavioral intervention revealed essentially the same results and was therefore excluded from the present analyses.

Table 2. Prediction of late cardiac events by pathogen burden-inflammatory marker indices

	Late cardiac event*		
	Relative Risk	95% CI	p
PB - CRP	4.16	1.20-14.39	0.02
Age	1.03	.95-1.1	0.49
Gender	1.99	.40-9.86	0.40
PB - Neopterin	3.76	1.00-13.99	0.05
Age	1.02	.94-1.11	0.59
Gender	1.14	.25-5.32	0.87

** 15 late cardiac events occurred, 163 patients had no recurrent cardiac event during the cardiac follow-up; PB= pathogen burden*

The standardized regression coefficients show a strong increase of the risk of PB when combined with CRP or neopterin (Table 3). The risk of PB almost doubles when combined with high levels of CRP. However, the risk of the systemic inflammation index is hardly larger than the risk of CRP alone. This suggests that the risk of the index that combines both PB and CRP is mainly due to the presence of CRP in that combination. The standardized regression coefficients also show a strong increase of the risk of PB when combined with neopterin. The risk of the PB and infection-induced inflammation index is substantially larger than the risk of neopterin alone. This indicates that the risk of CAD is increased in those patients, who have both evidence of seropositivity and infection-induced inflammatory activity.

Discussion

In the present study no direct associations were observed between future cardiac events and PB on the one hand and two markers of inflammation on the other hand. In contrast, we observed that the simultaneous presence of high PB and elevated levels of CRP or neopterin increase the risk of new cardiac events in PCI patients.

The major weakness of this study is formed by the strict inclusion criteria, which resulted in a study population that consisted of exhausted PCI patients. For the serological study this might be a disadvantage. Exhaustion is associated with inflammation and is an independent risk factor for CAD, which might have resulted in skewed distributions in contrast to non-exhausted PCI patients [23, 24]. A result of the inclusion criteria is a restriction of range. Furthermore, the low statistical power caused by the small number of new cardiac events is a weakness. This may explain the absence of a direct association between PB, CRP and neopterin with future cardiac events. Therefore, this observation does not refute the results of those studies that observed an unconfounded and direct association of PB or inflammation with CAD [6-9, 14]. Despite of the low statistical power we observed that the simultaneous presence of high PB and elevated inflammation increase the risk of new cardiac events. Does this observation support the contention by Zhu that pathogens play a causal role in the pathogenesis of atherosclerosis [12]?

Table 3. Standardized regression coefficients of the tested variables for late cardiac events

	Standardized Relative Risk*
PB	0.24
CRP	0.42
Neopterin	0.24
PB - CRP	0.44
PB - Neopterin	0.37

** A standardized coefficient represents the effect of an explanatory variable when this variable is expressed in units of its own standard deviation*

As shown in table 3 the standardized regression coefficient of late cardiac events and PB increases by 83% when combined with CRP. However, the standardized regression coefficient of this association is only 5% larger than the standardized regression coefficient of CRP alone. This strongly suggests that the risk of the PB-CRP combination has to be attributed to the presence of CRP in that combination. CRP is a marker of systemic inflammation. A high concentration of CRP could also be caused by inflammation that is not related to pathogens or by non-infectious inflammatory conditions [25]. CRP has been found to increase the risk of CAD in several studies [21, 26]. Thus, the observation that a combination of high PB and CRP increases the risk of new cardiac events gives little support to the contention that pathogens play a causal role in the pathogenesis of CAD.

Table 3 also shows that the standardized regression coefficient of late cardiac events and PB increases by 54% when combined with elevated neopterin. The table also shows that the standardized regression coefficient of neopterin increases by 54% when combined with high PB, suggesting that both PB and neopterin contribute to the risk of this combination. The elevated risk associated with this combination is plausible from a biological point of view. Neopterin is produced by activated monocytes and macrophages exclusively upon stimulation by IFN-γ [16]. Activation of monocytes and macrophages is critical in the development of CAD [27]. Different studies have demonstrated that neopterin levels are associated with coronary diseases [28-30]. A high PB does not tell anything about the activity of the pathogens included in the PB-index. It is the combination that counts. Therefore, this observation gives support to the contention of Zhu that pathogens are not innocent bystanders, but, when activated, do play a causal role in the pathogenesis of CAD.

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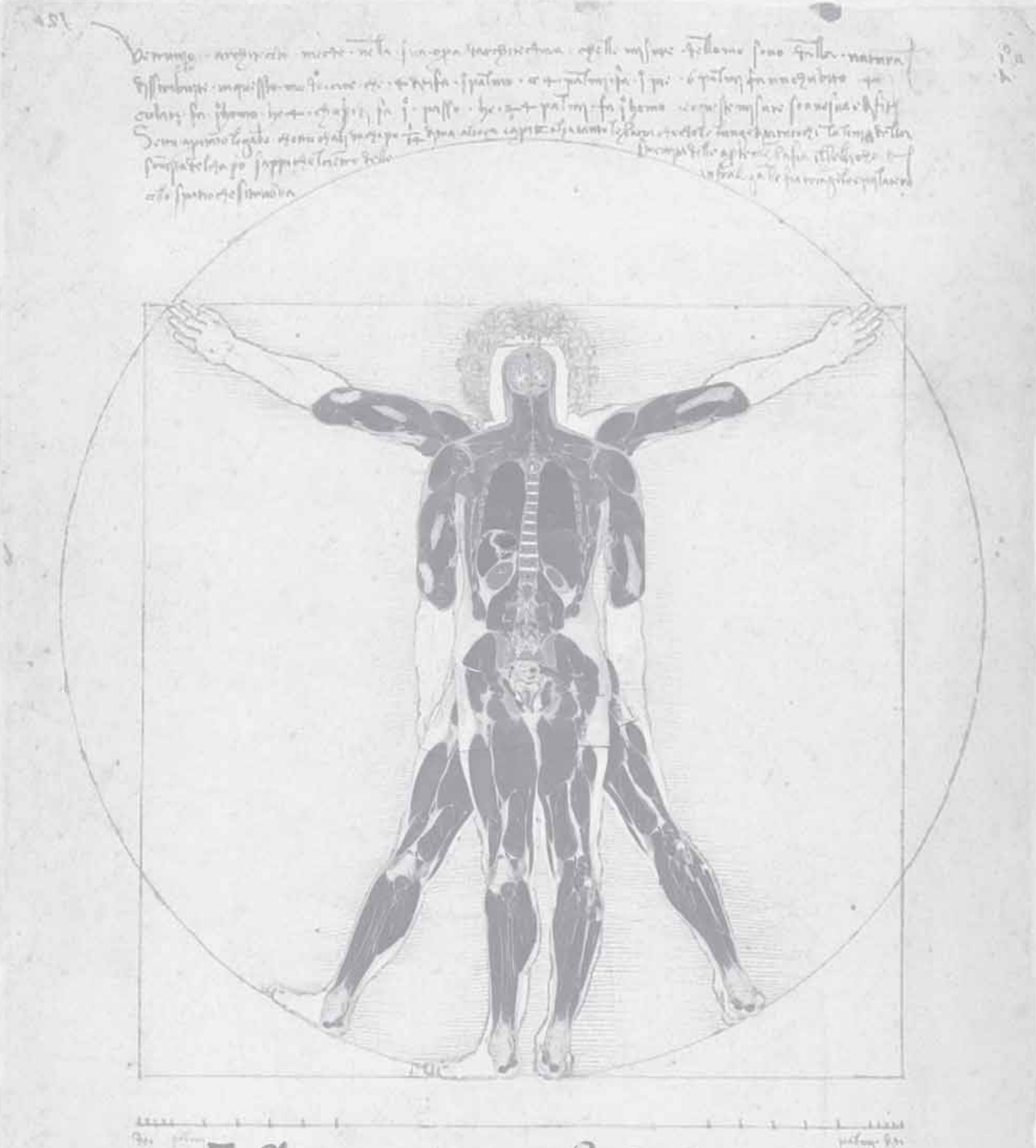
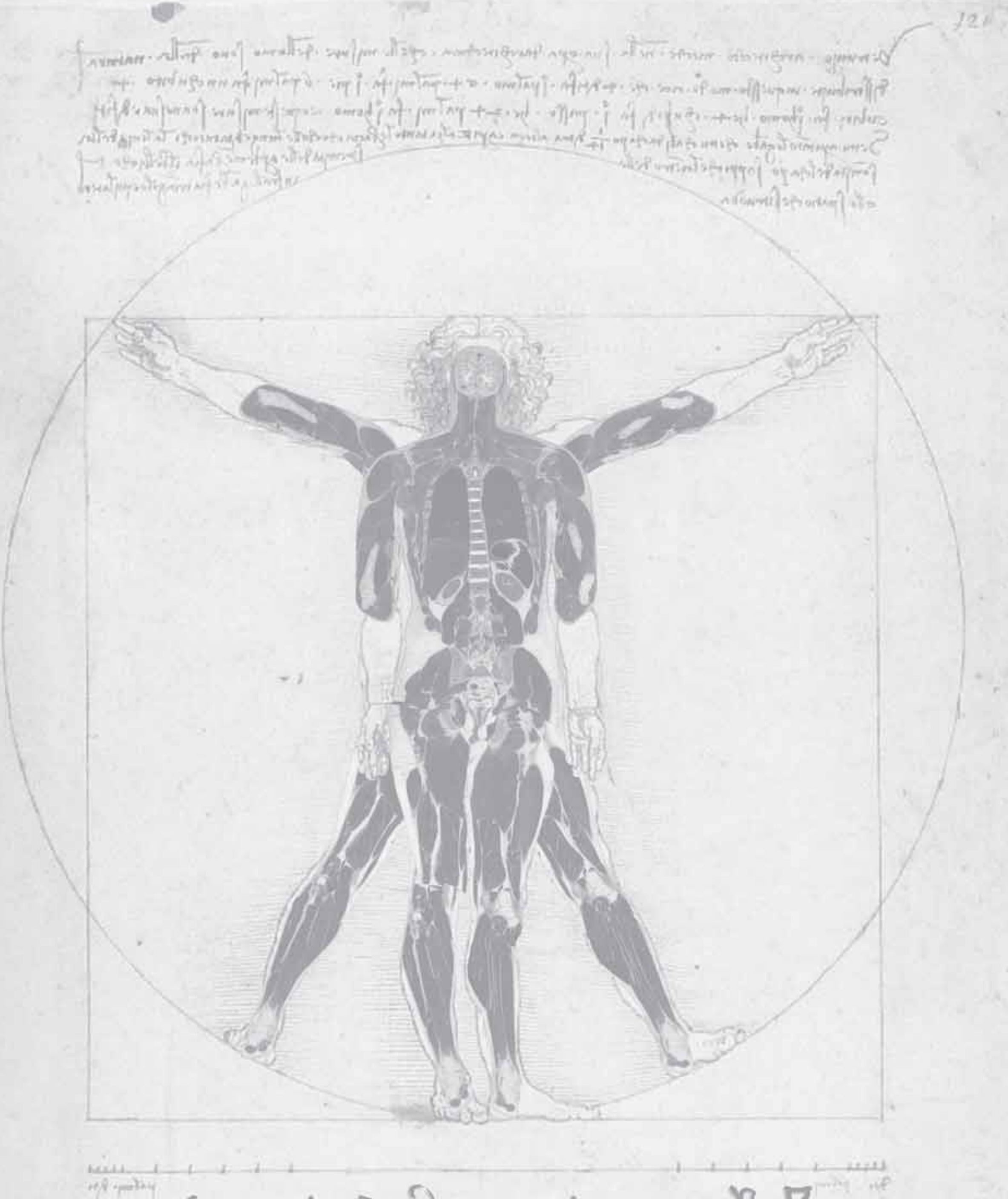


Inflammation, exhaustion and coronary artery disease

Modifiable psychobiological pathways

Martijn Kwaijtaal



Inflammation, exhaustion and coronary artery disease

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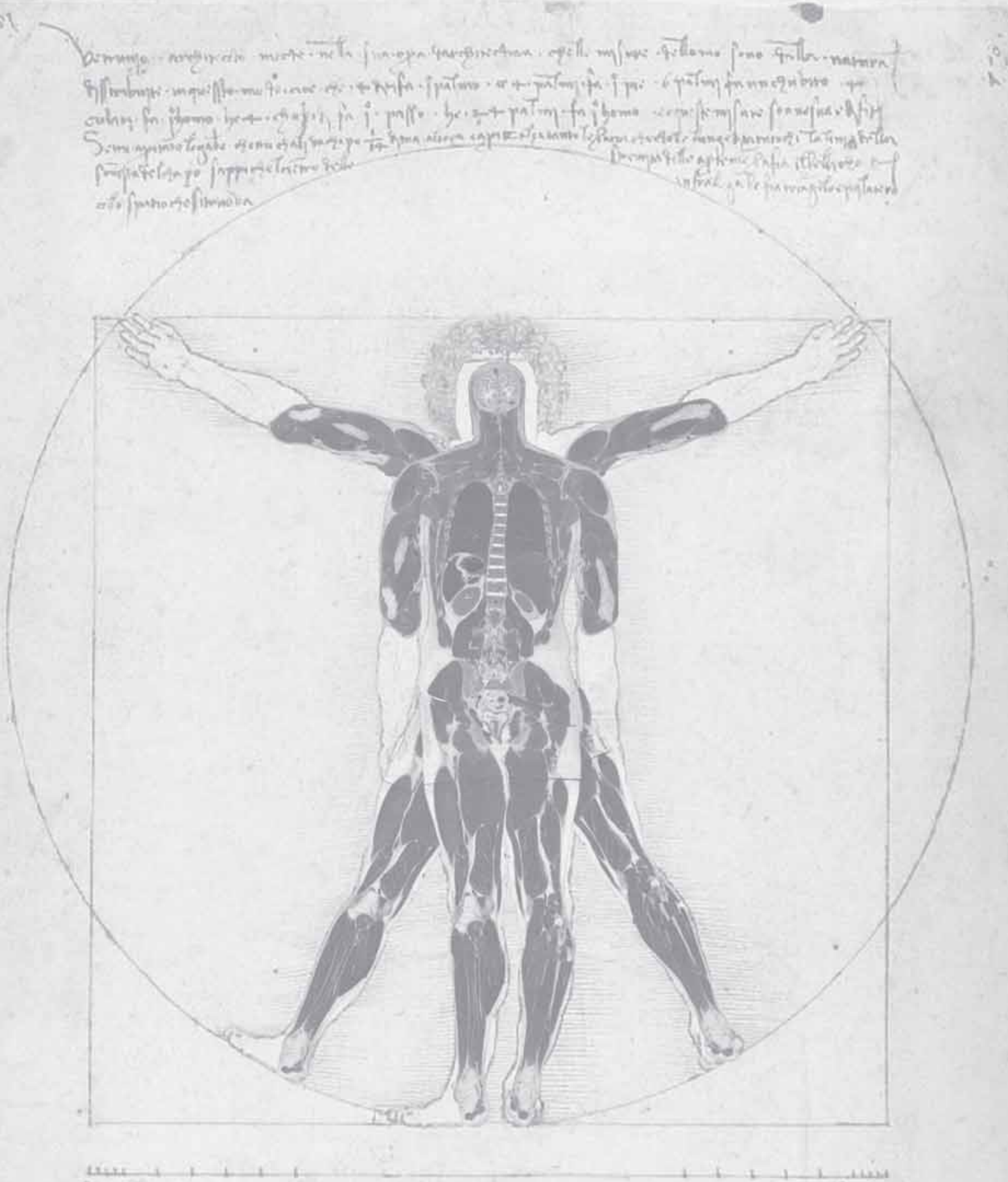
Maartje Kwaijtaal



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7

General discussion

General Discussion

CAD is the result of severe atherosclerosis, an inflammatory disease. The studies described in this thesis focused on underlying mechanisms of CAD and exhaustion. The general question was whether the depressive symptomatology that precedes CAD in more than half of all cases, approached in this thesis as a state of exhaustion, fosters inflammation.

Summary of the results

The main positive finding in these studies was that the behavioral intervention had a beneficial effect on inflammation. This beneficial effect was at least partly mediated by an increased nervus vagus activity. The main negative outcome of these studies was that there was no direct association between changes in exhaustion and changes in inflammation. In addition to these findings, we found that high concentrations of a number inflammatory markers increased the risk of late cardiac events. MIF was associated with exhaustion. Although we were not able to disclose the origin of MIF, the results fit into the contention that the HPA axis is hypoactive in exhausted subjects. Finally, we found that a combination of pathogen burden and inflammation has a predictive value.

Interpretation of the results

In contrast to other studies, we did not find associations between markers of inflammation and / or pathogens and exhaustion except for MIF. There are several possible reasons why we did not find any associations. Firstly, the study population of exhausted PCI patients makes these studies unique but also makes it harder to find associations because all patients were exhausted. A result of this inclusion criterion is a restriction of range. At 6 and 18 months a number of patients were not exhausted, however, we were still unable to find associations between changes in exhaustion and changes in inflammation.

Secondly, the process of selecting patients for the intervention and control group was approximately six weeks. Therefore, first blood collection was also approximately six weeks after PCI. The increases in inflammatory markers after PCI are transient and return to pre-procedural concentrations within a week and remain at pre-procedural concentrations up to at least one month after PCI [1]. It can therefore be argued that post-procedural concentrations of inflammatory markers, measured in blood approximately 6 weeks after index PCI, reflect the inflammatory status before the PCI because this procedure does not remove the inflammatory lesion. In an earlier study by Appels et al [2], blood samples were taken a day prior to PCI, it was shown that exhausted PCI patients expressed higher concentrations of inflammatory markers than non-exhausted PCI patients. The study also showed that exhausted PCI patients expressed higher titers of IgG antibodies to *Chlamydia Pneumoniae* and cytomegalovirus than non-exhausted PCI patients. It must be stated that the study used extreme groups of exhausted versus non-exhausted. Patients with moderate exhaustion were excluded. Appels et al also found an association between major depression (3 exhausted patients were also depressed) and inflammatory markers and IgG titers. In the sub-studies described in this thesis only exhausted PCI patients were included which hampered the replication of the results of Appels et al. In the sub-studies we also did not find any associations between depression and inflammatory markers and measures of serology.

Thirdly, patient numbers differed between the various sub-studies. At the start of the study, blood was collected of 252 consecutive patients in the Maastricht sample of the EXIT study population. Of these 252 patients 18 patients were excluded because of the prescription of immunosuppressive medication (i.e. medication exclusively developed to suppress the immune system). The patient sample for the sub-study in chapter two was 234, because at the start of blood collection only serum samples were collected which restricted the analyses of inflammatory mediators (there was not sufficient blood to analyze other possible mediators, such as IgG's and MIF). The number of patients with a HRV measurement was 249, of these 249 patients only 168 had both HRV and immunological measurements. Although a population of approximately 200 patients is substantial, during the statistical analyses power problems did occur.

Finally, because a number of samples were stored for more than 12 months in -20°C, a question arose about the quality of the serum samples used in the studies because a number of samples were stored for more than 12 months in -20°C. Several studies have shown that inflammatory mediators and other proteins can decay after a certain time. We have reasons to believe that these concerns are not justified for our studies. Firstly, all samples were collected and stored following a strict protocol. We used the same conditions and ELISA kits as Kenis et al [3]. That study showed that blood samples can be stored at -20°C for more than 12 months without substantial decay. Secondly, a pilot study was undertaken to ensure that serum could be used. We took 40 random samples of exhausted PCI patients and tested both plasma and serum samples of these patients (samples taken at the same time). The measurements were done in duplicate and plasma and serum samples were compared. Most samples were within 10% (plasma versus serum), a few serum samples were higher than plasma samples and a few plasma samples were higher than serum samples. Overall, plasma and serum did not differ within patients. There was also no difference between samples that were in storage for longer than 12 months, this was also shown in chapter 2. The markers of inflammation were remarkably stable over 18 months of follow-up. In sum, the integrity of our immune data is supported by the following considerations: a) all samples were treated following a standardized protocol, b) the pilot study using 40 different samples of both plasma and serum showed no significant differences between the use of plasma or serum and c) due to a possible decay an underestimation of the results is possible. An underestimation of the inflammatory marker concentrations could lead to an underestimation of the relative risks and an underestimation of the effect of the behavioral intervention.

The results of these studies provide insight in the biology of stress. Inflammation is a healthy response to tissue damage and injury. In exhausted subjects the response to injury is activated, but less inhibited, which can lead to (if not already present) a chronic inflammatory status. A behavioral intervention as used in EXIT was successful only in patients without a history of CAD and in patients without a chronic painful condition. Taken together a group of CAD patients with exhaustion, without a history of CAD and without a chronic painful condition is quite possibly better off being treated by a combination of PCI, followed by a behavioral intervention, which is aimed at reducing the feelings of exhaustion.

In conclusion, the EXIT study gives mixed support to the general hypothesis that the depressive symptomatology that precedes CAD in more than half of all cases, approached in this thesis as a state of exhaustion, fosters inflammation. The observation that the behavioral intervention had a beneficial effect on the early inflammatory response as reflected by neopterin and IL-1ra is of major theoretical and clinical importance. Further cues of the effect of the behavioral intervention on increases in HF HRV and therewith nervus vagus activity, is also an important observation. The beneficial effect of the behavioral intervention might at least partly have been mediated by increases in nervus vagus activity. However, the absence of unambiguous associations between (changes in) markers of inflammation and exhaustion raise questions about the value of the model and future research is necessary.

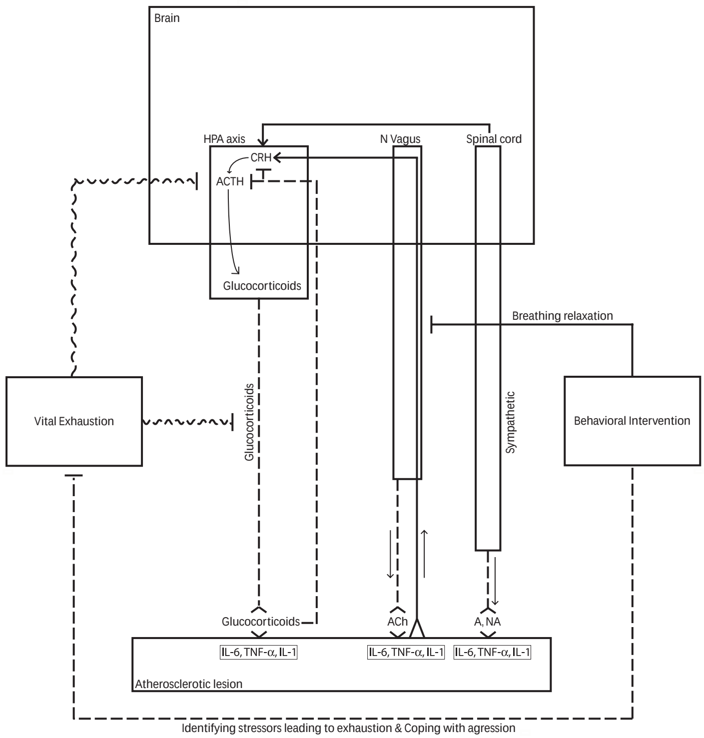
Suggestions for future research

There are several possible biological pathways by which the behavioral intervention in EXIT succeeded in decreasing the feelings of exhaustion. The behavioral intervention was a combination of identifying stressor leading to exhaustion, coping with hostility as one of the causes of exhaustion and recovery by making rest more efficient [4]. Most likely the combination of identifying and coping with stressors and hostility had a direct effect on the feelings of exhaustion, whereas the breathing and relaxation component most likely had its effect on the nervus vagus [5]. In Figure 1, the behavioral intervention was added to the model of "exhaustion, immune system and CAD" as presented in the general introduction. Central to the association between exhaustion and CAD is the HPA axis, and it would be of interest to test the HPA axis activity of both exhausted and non-exhausted PCI patients. Baseline measurement would ideally be before the PCI itself with monthly follow-up measurements. Very important in studies measuring HPA axis hormones is the time of blood collection, which needs to be at the same time during the morning for all patients and samples. By measuring the HPA axis activity in PCI patients and combining this with exhaustion, the association between exhaustion and changes in activity of the HPA axis can be determined and visa versa. In addition to hormones of the HPA axis that can be measured (e.g. CRH, ACTH and glucocorticoids), other inflammatory mediators need to be measured in order to replicate the findings reported in this thesis, specially MIF. By adding different biological parameters the whole inflammatory process can be monitored in combination with HPA axis activity, which should result in new insights of the role of chronic stress in the development of CAD with possible intervention techniques as a result.

Exhausted patients suffer from a hypoactivity of the HPA axis [6-10], therefore studies interfering with the HPA axis activity (i.e. stimulation of the HPA axis) should be considered as a possible treatment for exhaustion. Stimulation of the nervus vagus results in increased production of HPA axis hormones and therewith stimulation of the HPA axis (Figure 1). In the results in this thesis breathing relaxation is reported as a possible mechanism through which the behavioral intervention directly stimulated the nervus vagus. Future research should investigate to what extent breathing relaxation increases nervus vagus activity and to what extent this affects the HPA axis.

A different research approach is via vagal activity studies, these can be performed using direct stimulation of the nervus vagus by vagus nerve stimulation (VNS) therapy. In the treatment of epilepsy, VNS is common. A device, much like a pacemaker, is implanted and connected to the nervus vagus and electrical pulses stimulate it (reviewed by Groves and Brown [11]). In addition to epilepsy, trials are undertaken to use VNS to treat depression, anxiety and migraines. By stimulation of the nervus vagus, the HPA axis is stimulated to produce CRH, ACTH and glucocorticoids. Because the HPA axis is hypoactive in exhausted subjects, a stimulation of the nervus vagus might have beneficial effects on exhaustion and the pro-inflammatory state of exhausted subjects.

Figure 1. Interactions between brain and an atherosclerotic lesion in exhausted PCI patients undergoing the behavioral intervention in EXIT



The behavioral intervention (most likely the breathing relaxation component) has a beneficial effect on the nervus vagus activity which results in a) inhibition of pro-inflammatory mediators in the lesion by increased acetylcholine release in the lesion and b) stimulation of the HPA axis which results in more glucocorticoid production which results in more inhibition of pro-inflammatory mediators in the lesion. The behavioral intervention also results in a reduction of the feelings of exhaustion in these patients, but the direct pathways of these effects of the behavioral intervention on vital exhaustion are not known (but most likely the result of the coping with aggression and stressors leading to exhaustion).

In animal studies nervus vagus stimulants are used to pharmacologically stimulate the cholinergic anti-inflammatory pathway [12-15]. If agents like CNI-1493 can be used in RCTs the effect of this agent and agents of its sort can be associated with exhaustion. The HPA axis and changes in HPA axis activity can be monitored in these patients in addition to the inflammatory mediators that are involved in the process of atherosclerosis. Both VNS and drug therapy to enhance the activity of the HPA axis are interesting strategies to study the development and progression of atherosclerosis in combination with exhaustion.

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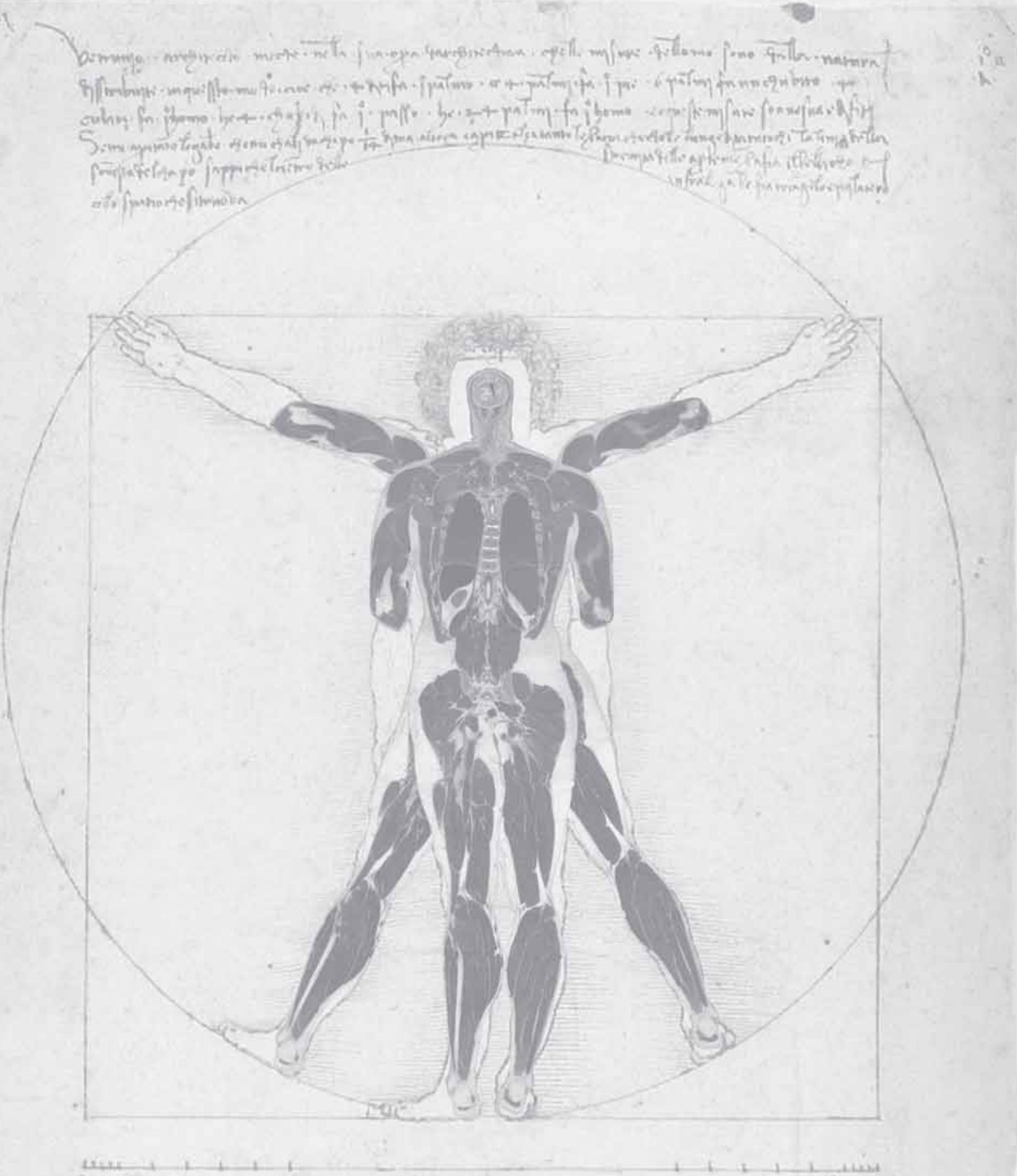

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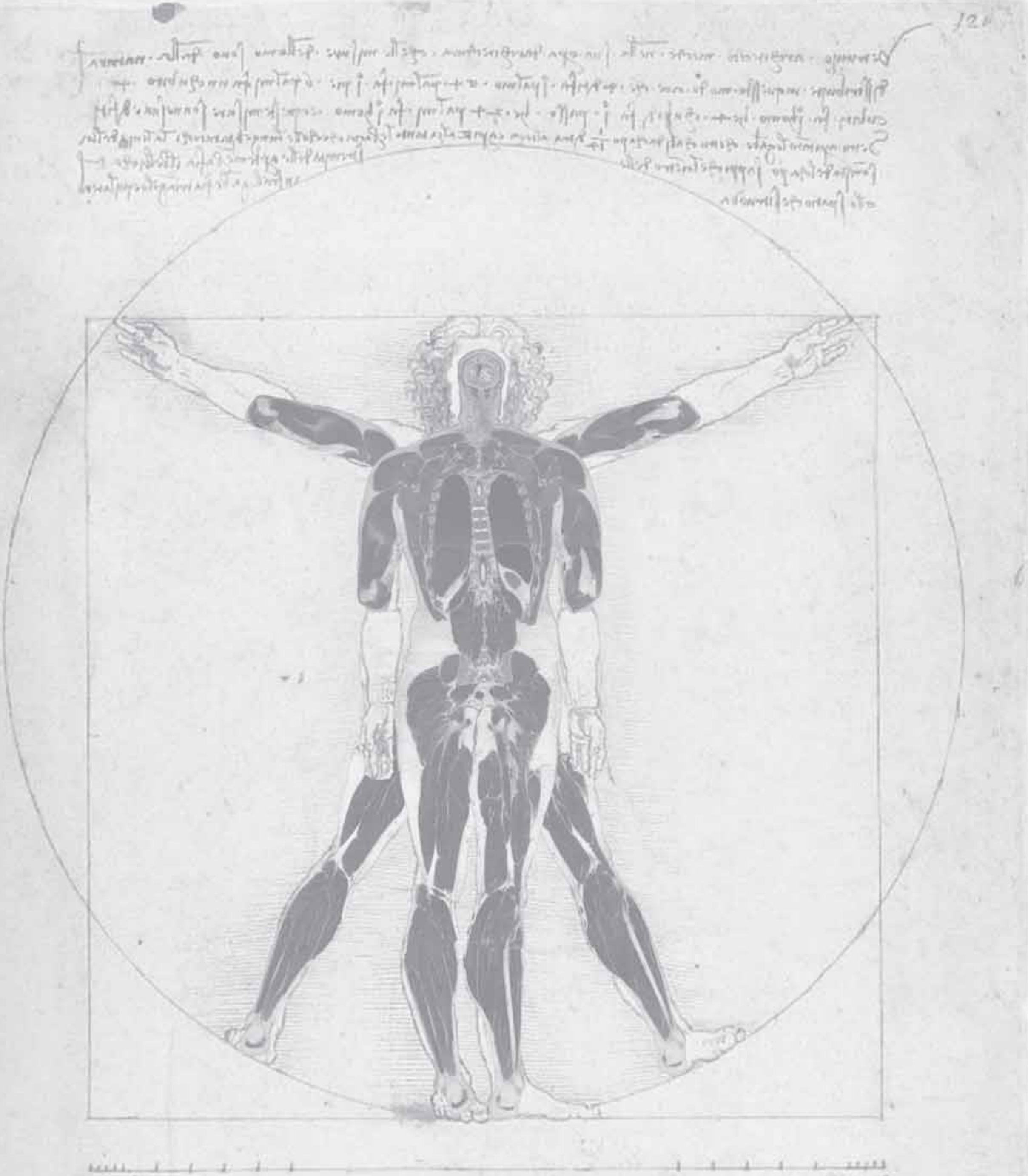

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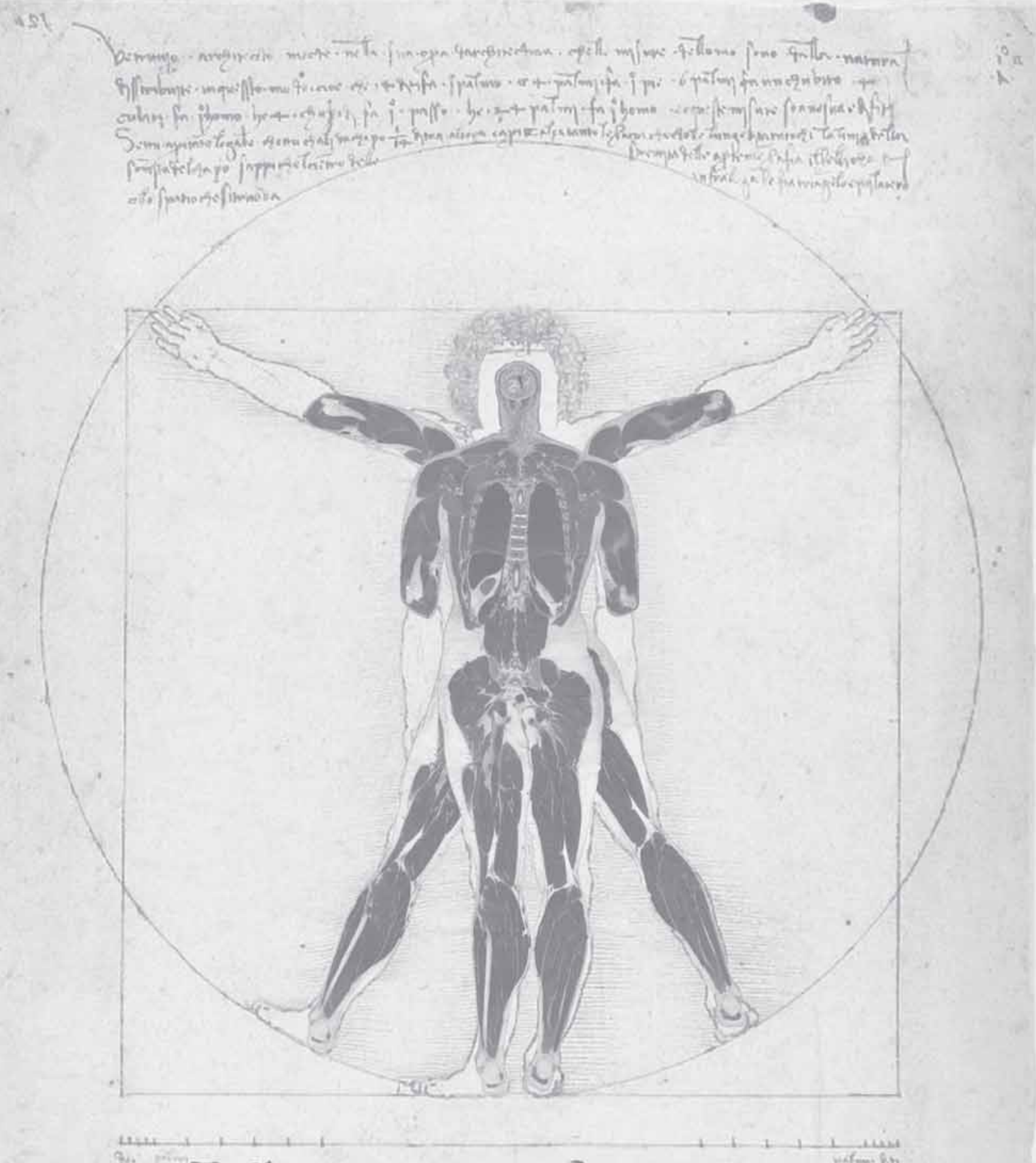

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Summary

Summary

Excess fatigue, listlessness and feelings of general malaise, hereafter exhaustion, increases the risk of a first cardiac event in a healthy population. In addition, the risk of a new cardiac event is increased in exhausted PCI patients. Previous research suggests that activation of immune-mediated inflammation may underlie the association between exhaustion and CAD. In the Exhaustion Intervention Trial (EXIT) exhausted PCI patients underwent a behavioral intervention to reduce the feelings of exhaustion. The second objective was to reduce the risk of a new cardiac event in these patients. Occurrence of new cardiac events (defined as re-PCI, coronary artery bypass graft surgery (CABG), myocardial infarction (MI) or cardiac death) was assessed through inspection of the medical records 24 months after inclusion. The time span of the behavioral intervention was 6 months and therefore cardiac events were divided in "early" cardiac events (i.e. events occurring within 6 months) and in "late" cardiac events (i.e. events occurring after 6 months). EXIT succeeded in reducing the feelings of exhaustion. In patients without a history of CAD and without a chronic painful condition the behavioral intervention reduced the risk of a late cardiac event.
In the sub-studies of EXIT, as described in this thesis, the general research question was whether the depressive symptomatology that precedes CAD in more than half of all cases, approached in this thesis as a state of exhaustion, fosters inflammation. The main objective was to investigate which biological mechanisms, which precede CAD, are mediated by the behavioral intervention.

The general research question was broken down in five research questions. The first research question was: "Is a state of exhaustion associated with inflammation?" In chapter two, this question was addressed. It is supposed that a decreased activity of the HPA axis underlies the association between a state of exhaustion and CAD observed in many epidemiological studies. CAD is an inflammatory disease and hypoactivity of the HPA axis activated immune-mediated inflammation. Appels et al observed positive associations between several markers of inflammation and exhaustion. This confirmed the psychophysiological model that underlies the theory that a state of exhaustion is a risk factor of CAD. This model was only partially supported by the EXIT study. It could be shown that the behavioral intervention reduced elevated concentrations of neopterin and IL-1ra. However, in contrast to the study by Appels et al no direct correlation between any marker of inflammation and exhaustion was observed. The results of the EXIT study confirmed the importance of inflammation in the pathogenesis of CAD. High concentrations of IL-6, IL-10 and CRP at baseline, and high concentrations of IL-6 and IL-1ra at 6 months increased the risk of late cardiac events in these patients. Remarkably, high concentrations of the anti-inflammatory mediator IL-10 also increased the risk of late cardiac events. This was most likely due to the pro-inflammatory state on the one hand and contra-regulatory mechanisms that fail on the other hand.

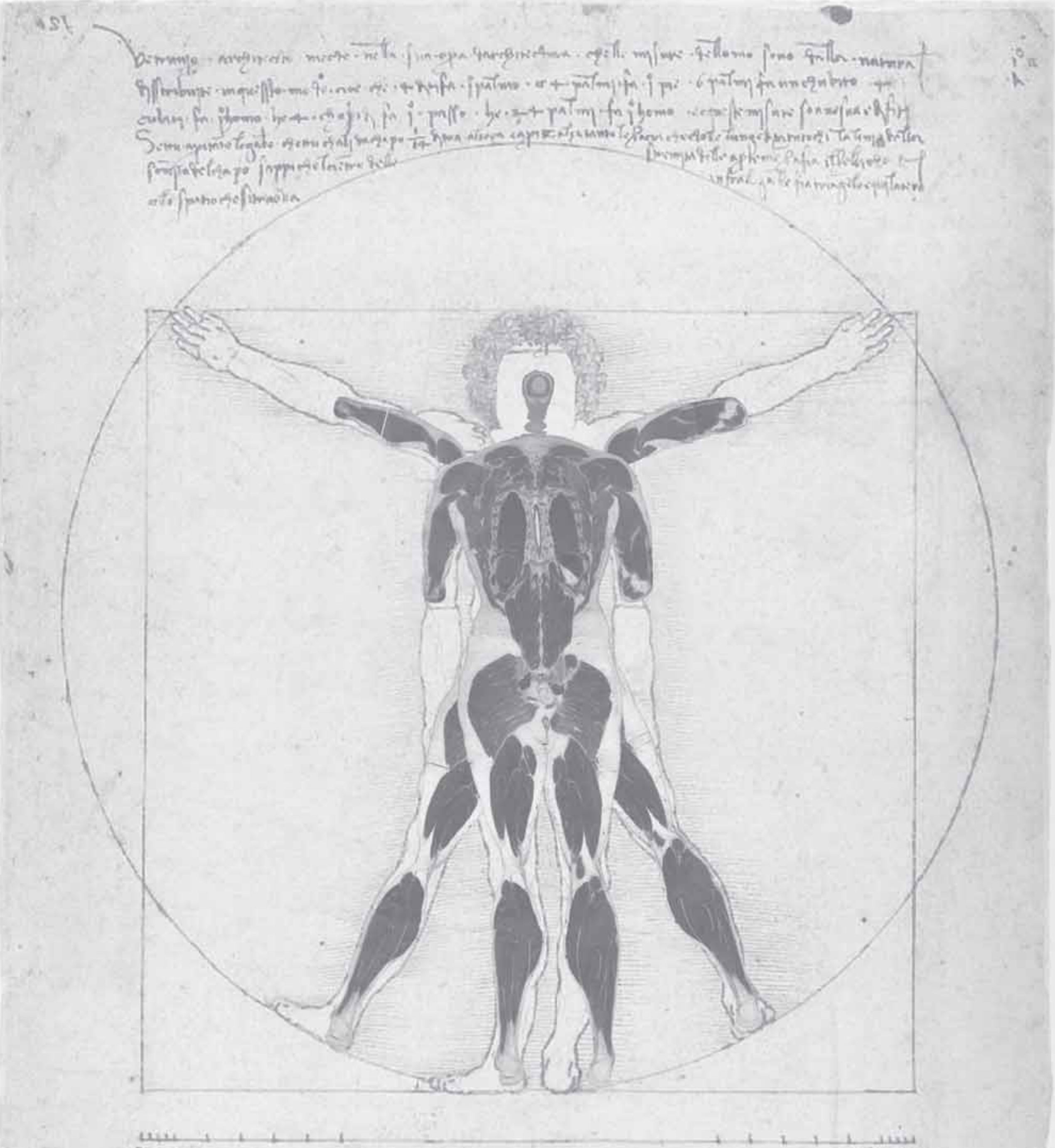
In chapter 3, the second research question was addressed: “Does a behavioral intervention aimed at the reduction of feelings of exhaustion have a beneficial effect on inflammation?” Results showed that the behavioral intervention reduced the odds of having an elevated concentration of neopterin at 18 months of follow-up by 68%. The behavioral intervention reduced the odds of having an elevated concentration of IL-1ra at 18 months of follow-up by 67% in those patients who did not suffer from a chronic painful condition (mostly rheumatic disease). The behavioral intervention had no effect on concentrations of TNF-α. Neopterin and IL-1ra are involved in the activation of monocytes and macrophages, one of the initial steps in the progression of atherosclerosis. Therefore, the results suggest that a behavioral intervention may contribute to the control of the inflammatory response, and possibly to the reduction of the risk of a coronary event. The beneficial effect of the behavioral intervention is most likely the result of stimulation of the nervus vagus. Stimulation of the nervus vagus has two main effects. It results in an increased production of acetylcholine, which results in an inhibition of neopterin and IL-1ra. The second effect is formed by a stimulation of the HPA axis, which results in an increased production of cortisol, which is a well known suppressor of inflammation.

The third research question was: “Does the behavioral intervention contribute to a decrease of the inflammatory status of PCI patients by increasing their vagal activity as reflected by HF HRV?” In chapter 4, this question was addressed. Autonomic functions (e.g. heart rate), which are normally under involuntary control, can be modulated by signals originating from higher brain centers. For instance, vagal control of heart rate (as reflected by the high-frequency (HF) component of heart rate variability (HRV)) can be enhanced through HRV biofeedback or paced breathing. When cytokine concentrations are low the CNS is informed about the inflammatory status by the ANS, in particular the afferent nervus vagus. Afferent nervus vagus activity can further generate a rapid anti-inflammatory response that is partly mediated by the efferent nervus vagus. The stimulated efferent nervus vagus releases acetylcholine that deactivates macrophages by inhibiting the release of TNF-α, IL-1 and other cytokines (the “inflammatory reflex”). This suggests, as formulated by Tracey, that intentional modulation of nervus vagus activity may provide a therapeutic advantage for inflammatory disease. The results of this study showed that the beneficial effects as found in chapter 3 were at least partly mediated by increases in HF HRV, which reflects vagal activity. This is one of the first studies to test Tracey’s “inflammatory reflex” model and show a possible mediating effect of the nervus vagus on inflammation and finally that the effect can be modified by the behavioral intervention.

The fourth research question was: “Is a state of exhaustion associated with a decreased activity of the HPA axis, as reflected by decreased concentrations of macrophage migration inhibitory factor (MIF)?” This question was addressed in chapter 5. MIF is a pro-inflammatory cytokine produced by cells of the immune system. Therefore, one may expect a positive association of MIF with PCI. This explorative study, however, showed that PCI patients displayed lower concentrations of MIF at baseline than an age-sex matched control (reference) group. MIF concentrations increased significantly over 18 months of follow-up in these PCI patients and returned to the concentrations observed in the reference group. The pattern of MIF expression differed from other inflammatory markers (which were stable). MIF concentrations were not associated with late cardiac events in PCI patients, although, a negative trend was observed for late cardiac events versus MIF concentrations. MIF is also produced in a hormone like fashion by the anterior pituitary, which is a part of the HPA axis. Therefore one may expect a negative association of MIF and exhaustion, because available evidence strongly suggests that the HPA axis is hypoactive in exhausted subjects. At 18 months of follow-up the MIF concentrations in the non-exhausted group were significantly higher than in the exhausted group. The results do not disclose the origin of MIF assessed in blood, however, these observations fit into the contention that the HPA axis is in exhausted subjects.

The fifth research question was: “Is a state of exhaustion associated with pathogen burden?” In chapter 6 this issue was addressed. Pathogens are suspected to play a role in the initiation and duration of inflammation. Van Der Ven et al. have shown that pathogen burden is positively associated with exhaustion, suggesting that pathogens may be (re)activated in a state of exhaustion. We were not able to replicate their findings, this might be due to the design of the study, which did not include patients who did not feel exhausted, and due to the stability of the IgG pathogen titers. The study showed a positive association between a combination of pathogen burden and inflammation on the one hand and the occurrence of late cardiac events on the other hand. We observed that those patients characterized by the simultaneous presence of high pathogen burden and elevated concentrations of CRP, or by the presence of high pathogen burden and elevated concentrations of neopterin, are at increased risk for a late cardiac event. Detailed analyses indicated that the predictive power of the first combination has to be attributed to the presence of CRP in that combination. The predictive power of the second combination that includes a marker of monocyte/macrophage activation supports the contention of Zhu that pathogens are not innocent bystanders in the development of CAD.

The results of these studies give insight into the biology of stress. Inflammation is a healthy reaction of the immune system to tissue damage and injury. In exhausted subjects the response to injury is activated, however, less inhibition is available which can result in (if not already present) a chronic pro-inflammatory status. The results of the EXIT study indicate that a behavioral intervention in PCI patients can reduce the risk of a second cardiac event. EXIT reduced the risk of a late cardiac event and reduced inflammation through increased nervus vagus activity.



Inflammation, exhaustion and coronary artery disease

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Martijn Kwaijtaal

Nederlandse Samenvatting

Nederlandse samenvatting

Gevoelens van ongewone vermoeidheid, verlies van energie en toegenomen prikkelbaarheid, hierna uitputting genoemd, verhogen bij gezonde personen het risico op een eerste cardiale gebeurtenis. Daarbij verhogen zij bij dotterpatiënten het risico op een tweede cardiale gebeurtenis. Eerder onderzoek suggereert dat dit verband tussen uitputting en hart- en vaatlijden berust op activatie van ontstekingsreacties. In de Exhaustion Intervention Trial (EXIT) is bij uitgeputte dotterpatiënten onderzocht of een psychologisch interventieprogramma de gevoelens van uitputting bij deze patiënten kan verminderen. Het tweede doel was om daaropvolgend het risico van een nieuwe cardiale gebeurtenis te verlagen. Het optreden van een nieuwe cardiale gebeurtenis (gedefinieerd als een nieuwe dotterbehandeling, een bypass operatie, hartinfarct of cardiale dood) werd 24 maanden na de start van EXIT vastgesteld door inspectie van de medische status. Aangezien het interventieprogramma 6 maanden duurde, werden cardiale gebeurtenissen opgesplitst in “vroege” gebeurtenissen (tijdens het programma) en “late” gebeurtenissen (na het programma). De gedragsinterventie was in staat om de gevoelens van uitputting terug te dringen. In patiënten zonder cardiale geschiedenis en zonder co-morbiditeit was de gedragsinterventie ook in staat om de kans op late cardiale gebeurtenissen te verminderen.

In de substudies van EXIT, zoals deze beschreven zijn in dit proefschrift, was de algemene vraag of de depressieve symptomatologie (d.w.z. uitputting) die in meer dan 50% van alle dotterpatiënten vooraf gaat aan hart- en vaatlijden, verbonden is met ontsteking. Het doel was om te onderzoeken welke biologische mechanismen, die aan de ontwikkeling van hart- en vaatlijden ten grondslag liggen, beïnvloed worden door de gedragsinterventie.

Om dit te onderzoeken werd het doel opgesplitst in 5 onderzoeksvraagstukken. De eerste onderzoeksvraag was: “Hangt een staat van uitputting samen met ontsteking?” In hoofdstuk 2 is deze vraag onderzocht. In een aantal epidemiologische studies wordt een verband gevonden tussen uitputting en coronaire vaatziekten, een mogelijke verklaring hiervoor is een verlaagde activiteit van de hypothalamus-hypofyse-bijnier (HPA) as in uitgeputte personen. Coronaire vaatziekten zijn ontstekingsziekten en een verlaagde activiteit van de HPA as kan leiden tot verhoogde immuun gereguleerde ontstekingen. Appels et al hebben aangetoond dat er een verband bestaat tussen uitputting en een aantal ontstekingsindicatoren wat het onderliggende psychofysiologische model van de staat van uitputting als risicofactor voor coronaire vaatziekten ondersteunt. Dit model werd echter maar gedeeltelijk ondersteund door de substudies van EXIT zoals deze beschreven zijn in dit proefschrift. Er werd aangetoond dat de gedragsinterventie in staat was om hoge concentraties van ontstekingsactiverende indicatoren terug te dringen. Echter, in tegenstelling tot de studie van Appels et al werd er geen direct verband gevonden tussen uitputting en ontstekingsindicatoren. De resultaten van EXIT laten zien dat ontstekingsreacties in de ontwikkeling van coronaire vaatziekten van groot belang zijn.

Hoge concentraties van IL-6, IL-10 en CRP bij het begin van de studie verhogen het risico op het krijgen van late cardiale gebeurtenissen (6 maanden na het begin van de gedragsinterventie). Hoge concentraties van IL-6 en IL-1ra op 6 maanden na het begin van de gedragsinterventie verhogen ook het risico op late cardiale gebeurtenissen. Hoge concentraties van IL-10, een ontstekingsremmende indicator, verhoogde ook het risico op het krijgen van late cardiale gebeurtenissen. Waarschijnlijk komt dit door aan de ene kant de aanwezigheid van een chronische ontsteking wat een chronische verhoging van ontstekingsindicatoren (zowel ontstekingsremmend als ontstekingsactiverend) tot gevolg heeft en aan de andere kant contra-regulatoire mechanismen die niet goed (meer) werken.

In hoofdstuk 3 werd het tweede onderzoeksvraagstuk: “Heeft een gedragsinterventie gericht op het verminderen van de gevoelens van uitputting een remmende werking op ontsteking?” besproken. De resultaten lieten zien dat de gedragsinterventie de kans op hoge concentraties van neopterine op 18 maanden na het begin van de gedragsinterventie met 68% deed verlagen. De kans op hoge concentraties van IL-1ra op 18 maanden na het begin van de gedragsinterventie werd met 67% verlaagd in patiënten zonder een chronisch pijnlijke aandoening (meestal reuma). De gedragsinterventie had geen effect op TNF-α concentraties. Neopterine en IL-1ra zijn betrokken bij het activeren van monocyten en macrofagen, een van de eerste stappen in de vorming van aderverkalking. De gedragsinterventie kan op deze manier wellicht bijdragen aan het controleren van de inflammatoire respons en mogelijk tot het verlagen van het risico op het krijgen van nieuwe cardiale gebeurtenissen. Het positieve effect van de gedragsinterventie is waarschijnlijk het resultaat van nervus vagus activatie. Het activeren van de nervus vagus heeft namelijk twee belangrijke effecten. Ten eerste resulteert het in een verhoging van acetylcholine met als gevolg een remming van neopterine en IL-1ra. Het tweede effect is een stimulatie van de HPA as met als gevolg een verhoging van cortisol in het bloed. Cortisol is een bekend stresshormoon dat ontsteking remt.

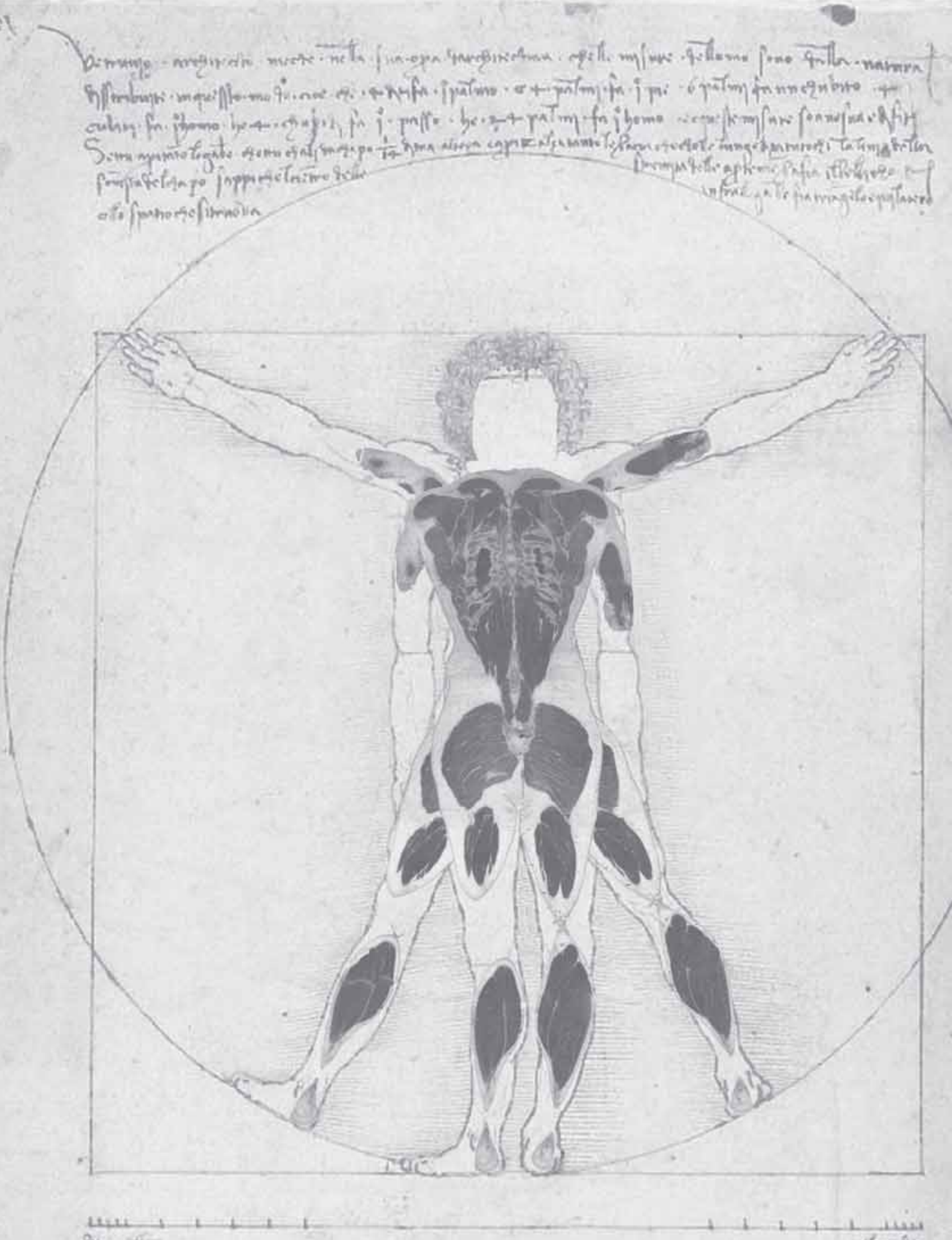
De derde onderzoeksvraag: “Draagt de gedragsinterventie bij aan het verminderen van ontstekingsreacties door een toegenomen activiteit van de nervus vagus zoals gemeten door de hoge frequentie hartritme variabiliteit?” werd in hoofdstuk 4 behandeld. Autonome functies (zoals hartslag), die normaal onder onvrijwillige controle staan, kunnen aangestuurd worden door dieper gelegen hersencentra. Controle over de hartslag gemediëerd door de nervus vagus (zoals gemeten door de hoge frequentie component van de hartritme variabiliteit) kan bijvoorbeeld versterkt worden door biofeedback van hartritmevariabiliteit of door aangepaste ademhaling. Wanneer concentraties van ontstekingsindicatoren laag zijn in het bloed, wordt er feedback gegeven aan het centrale zenuwstelsel door het autonome zenuwstelsel, voornamelijk via de afferente nervus vagus. De afferente nervus vagus kan een snelle ontstekingsremmende respons geven die voor een gedeelte wordt gemediëerd door de efferente nervus vagus.

De efferente nervus vagus zorgt voor een verhoging van acetylcholine dat macrofagen en monocyten deactiveert met als gevolg een stop in de release van TNF-α, IL-1² en andere cytokinen (de "inflammatoire reflex"). Dit wekt de suggestie dat een doelbewuste stimulatie van de nervus vagus mogelijk een therapeutische werking kan hebben op ontstekingsziekten. De resultaten van deze studie lieten zien dat de positieve effecten van de gedragsinterventie zoals beschreven in hoofdstuk 3, in ieder geval voor een gedeelte werd gemediëerd door een toename in hoge frequentie hartritme variabiliteit, wat overeen komt met een verhoogde activiteit van de nervus vagus. Dit is een van de eerste studies die het "inflammatoire reflex" model van Tracey test en daarnaast laat zien dat er een mogelijk mediërend effect bestaat van de nervus vagus en als laatste dat deze te beïnvloeden is door een gedragsinterventie.

In hoofdstuk 5 werd de onderzoeksvraag: "Hangt een staat van uitputting samen met een verlaagde activiteit van de HPA as, zoals gemeten door verlaagde concentraties van macrophage migration inhibitory factor (MIF)?" behandeld. MIF is een eiwit dat ontsteking bevordert en geproduceerd wordt door cellen van het immuunsysteem. Men zou daarom een positieve samenhang tussen PCI patiënten en MIF kunnen verwachten. Deze exploratieve studie liet echter zien dat PCI patiënten op baseline lagere concentraties van MIF hebben dan een gezonde, op leeftijd en geslacht geselecteerde controlegroep. Over een periode van 18 maanden na inclusie stegen de concentraties van MIF naar de hoogte zoals die ook gevonden werd bij de gezonde controlegroep. De MIF concentraties volgden gedurende de 18 maanden na inclusie een ander expressiepatroon dan andere ontstekingsindicatoren welke gelijk bleven. Verder werd gevonden dat er in PCI patiënten met lage concentraties van MIF op baseline twee keer zoveel nieuwe cardiale gebeurtenissen optraden als in PCI patiënten met de hoogste MIF concentraties. MIF wordt ook aangemaakt door de hypofyse, een onderdeel van de HPA as. Aangezien bekend is dat er bij uitgeputte personen een verminderde werking is van de HPA as, zou men kunnen verwachten dat er bij uitgeputte personen ook lagere concentraties van MIF zijn. Uitgeputte patiënten hadden op 18 maanden na inclusie hogere concentraties van MIF in het bloed dan niet uitgeputte patiënten. De resultaten van deze studie stellen ons niet in staat iets te zeggen over de herkomst van het MIF zoals wij deze gemeten hebben in de plasma monsters. Deze observaties passen echter in het beeld dat de HPA as een verminderde werking heeft in uitgeputte personen.

De vijfde en laatste onderzoeksvraag: "Is een staat van uitputting geassocieerd met pathogen burden?" werd in hoofdstuk 6 behandeld. Van pathogenen wordt verwacht dat ze een rol spelen bij de initiatie en duur van ontstekingsreacties. Van der Ven et al hebben aangetoond dat pathogen burden een positieve samenhang heeft met uitputting, wat aangeeft dat pathogenen wellicht worden ge(re)activeerd door uitputting. Helaas waren wij niet in staat om deze resultaten te reproduceren, wellicht door het design van de studie. Het design liet alleen uitgeputte dotterpatiënten toe, wat een restriction of range effect tot gevolg had. Daarnaast zijn IgG titers erg stabiel over tijd. De studie toonde aan dat er een positieve samenhang is tussen patiënten met zowel een hoge pathogen burden en hoge concentraties van CRP en hoge pathogen burden en hoge concentraties van neopterine en dit in relatie tot het optreden van nieuwe cardiale gebeurtenissen. Gedetailleerde analyses toonden aan dat het voorspellende vermogen van de eerste combinatie voornamelijk werd verklaard door de aanwezigheid van CRP. Het voorspellende vermogen van de tweede combinatie die neopterine bevat, een indicator van monocyten/macrofagen activatie, zet de bewering van Zhu dat pathogenen geen onschuldige omstanders zijn in de ontwikkeling van aderverkalking kracht bij.

De resultaten van deze studies geven inzicht in de biologie van stress. Ontstekingsreacties zijn een gezonde reactie van het immuunsysteem op weefselschade en letsel. In uitgeputte personen is de reactie op letsel geactiveerd, maar er zijn minder remmingen, wat kan leiden tot (als deze situatie niet al aanwezig is) een chronische staat van ontsteking. De resultaten van de hele EXIT studie geven een sterke indicatie dat een gedragsinterventie bij dotterpatiënten kan bijdragen aan het verminderen van het risico op een tweede cardiale gebeurtenis. EXIT verminderde de kans op een late cardiale gebeurtenis en reduceerde ontsteking door een verhoging van de nervus vagus activiteit.



Inflammation, exhaustion and coronary artery disease

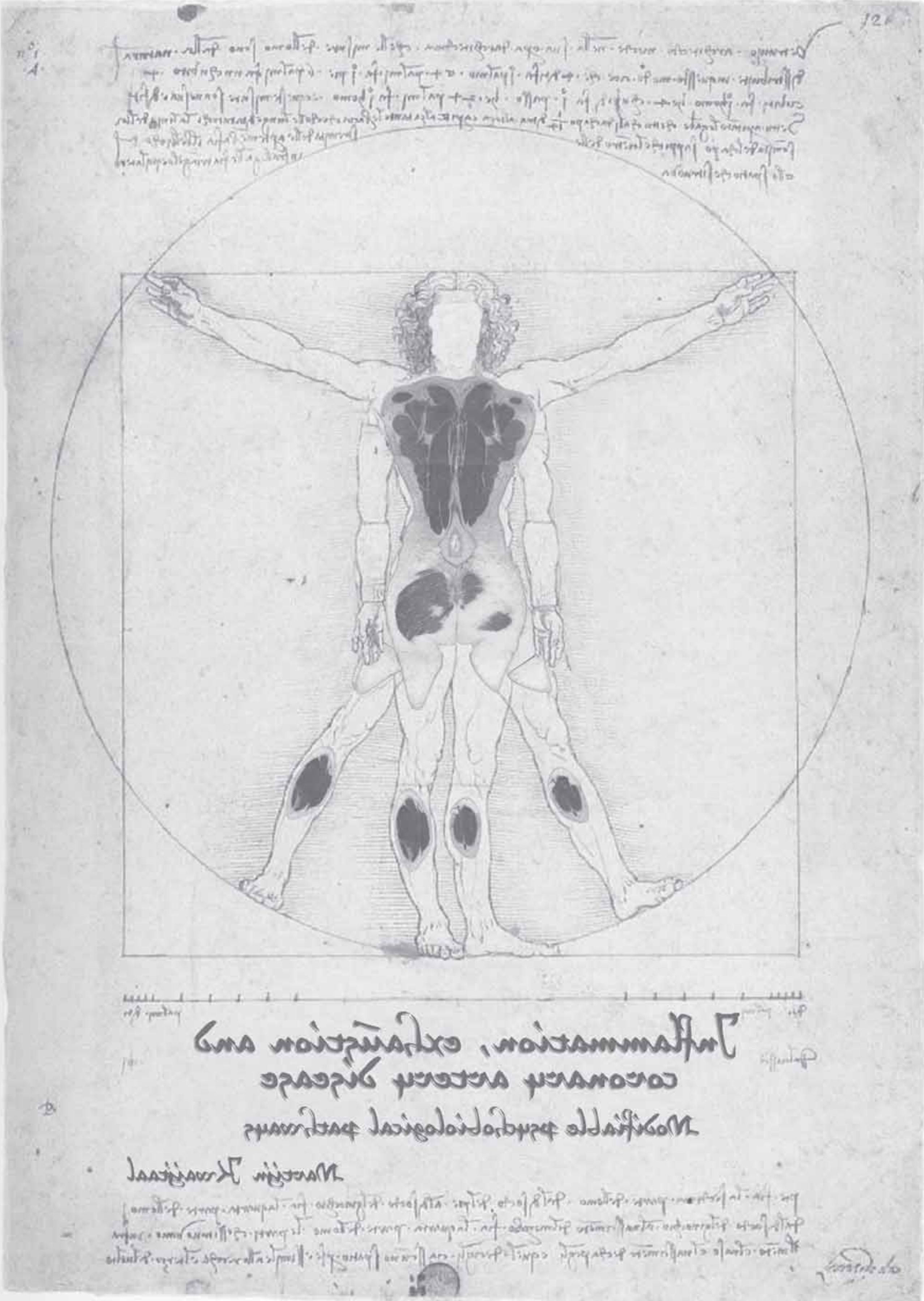
Modifiable psychobiological pathways

Martijn Kwaijtaal

Curriculum Vitae

Curriculum Vitae

Martijn Kwaijtaal was born May 25th 1976 in Wahroonga, NSW, Australia. In early life his parents moved back to the Netherlands. Growing up in a little village south of Breda, Martijn decided to study Biological Health Sciences in Maastricht after finishing his secondary school in 1995. Maastricht University offered Martijn options to explore more than just the study of Biological Health Sciences. He organized events for 2000 fresh students and worked in the ICT department during his study as database engineer and system operator. As the end of the study approached, a combined internship at the department of Neuropsychology and Psychobiology of Maastricht University and the department of Anatomy and Radiology at the university of Auckland, New Zealand for a year resulted in the thesis for his Masters degree and a publication in Neuroscience. He graduated in August 2001. After finishing the degree, Martijn went to work for the ICT department of the Maastricht University once again. This is where he met Rob van Diest, which eventually resulted in an appointment as junior researcher for two years, starting in June 2002. The project funded by the Netherlands Heart Foundation produced so much data and possibilities that in 2004 additional funding was requested and granted for another year in order for Martijn to write his dissertation. Martijn received a Scholarship Award in 2005 by the American Psychosomatic Society for the abstract: “Impact of a behavioral intervention on inflammation in coronary patients“. Currently, Martijn is working as data manager for the department of Psychology and Health at Tilburg University, the Netherlands.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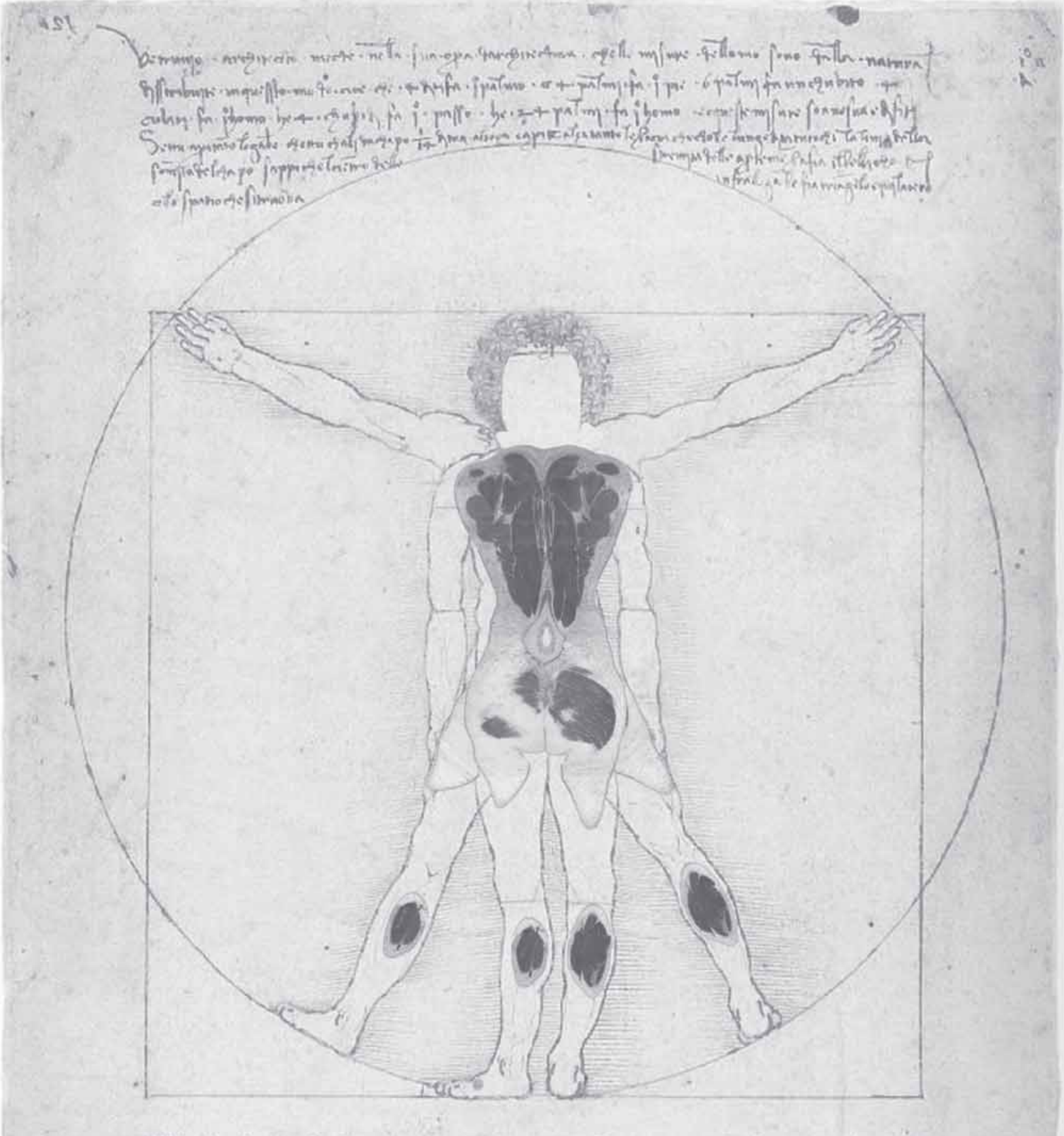

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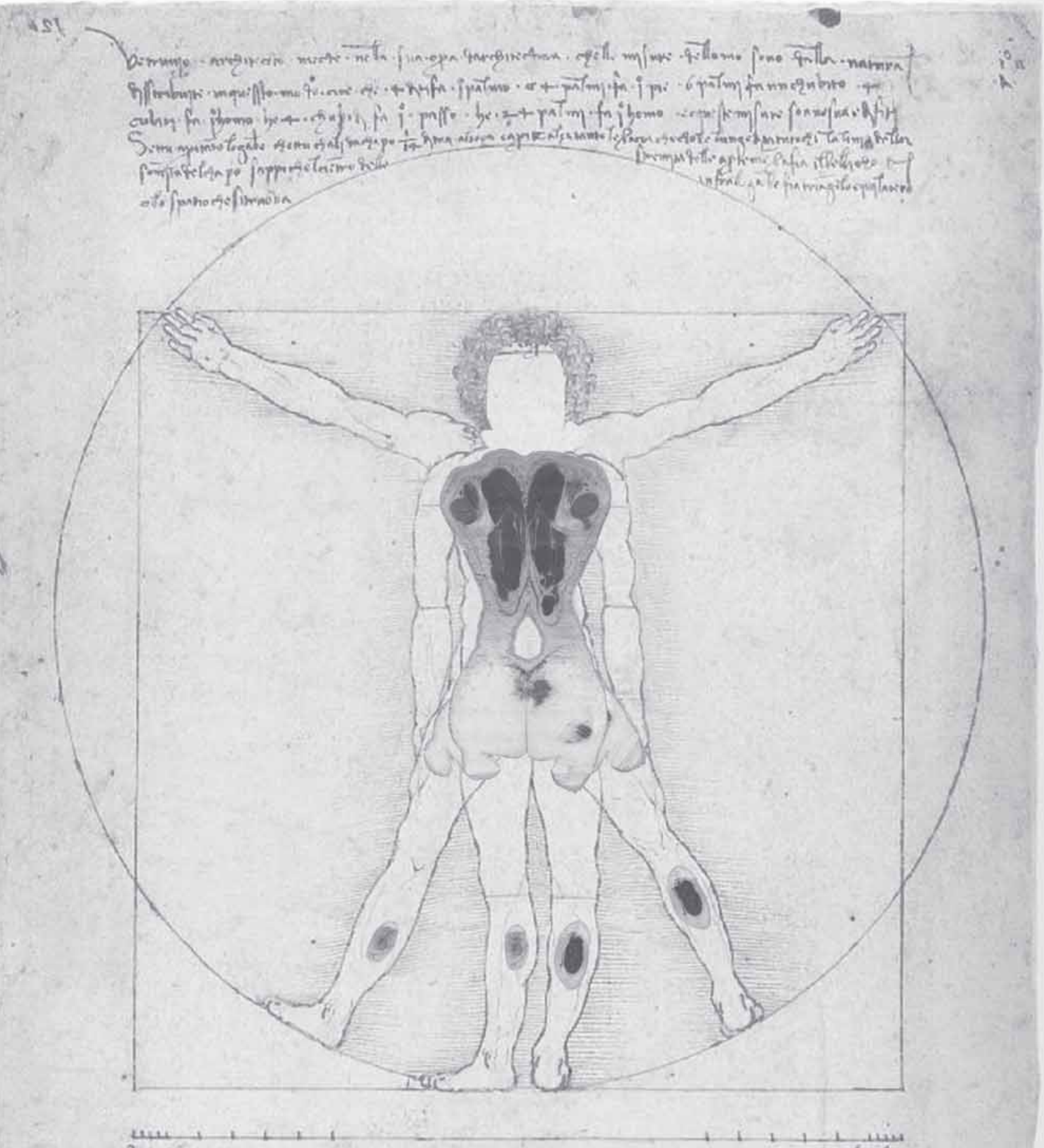

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Martijn Kwaijtaal

Dankwoord

Dankwoord

Dit boekwerk is tot stand gekomen door hulp van flink wat mensen, het is dan ook niet meer dan normaal om een aantal van deze mensen even te bedanken (helaas is het niet mogelijk om iedereen te bedanken). Relativeren is een kunst, echter was er 1 iemand die een tijdje geleden het relativeren misschien wel op het spits dreef... Henk stuurde mij een tijdje geleden een sms-je met daarin in de vraag: "zeg Kwaij, is dat knipselkrantje van je nou onderhand eens een keertje af?" Met regelmaat, als ik wat moeite had met schrijven of misschien toch wel een beetje gefrustreerd was over de snelheid waarmee het schrijfwerk vorderde moest ik weer aan die uitermate relativerende gedachte denken, het heeft me geholpen, toch wel dank daarvoor, denk ik...

Beste Ad, het is me een waar genoegen dat ik met je heb samen mogen werken! Ik voel me enorm vereerd dat ik je laatste promovendus mocht zijn in een enorme indrukwekkende reeks aan doctoren die je hebt voortgebracht. Ik ga onze bijeenkomsten enorm missen, een aantal dingen wist ik op voorhand zeker als ik een werkbespreking met jou en Rob had: 1. ik kom altijd wijzer uit deze bespreking, 2. ik heb weer een hoop werk te doen. En toch ging ik altijd met enorm veel plezier zowel de besprekingen in, als ook weer uit met een bak werk waar je u tegen zegt. Ik denk dat het onder andere de gedachte was dat ik er toch weer beter van zou worden dat het zo makkelijk en prettig maakte om met jullie samen te werken. No worries, we komen er wel!

Via Rob van Diest ben ik in contact gekomen met het onderzoeksproject. Rob was de hoofdaanvrager van de subsidie van de Hartstichting. Als jij niet met je kapotte computer binnen was komen struinen bij mijn voormalige werkgever (ICTS afdeling van de Universiteit Maastricht), dan was ik nooit aan dit dankwoord (lees proefschrift) begonnen… Ik heb altijd met enorm veel plezier met je samengewerkt, het was met name de vastberadenheid om alles tot in de puntjes perfect te maken dat ons op bepaalde momenten tot op het scherpst van de snede heeft doen discussiëren over zinsconstructies en woordkeuzes… Wellicht leek het er niet altijd even duidelijk op, maar zelfs op die momenten kon ik je aanwezigheid nog enorm waarderen, ik heb er in ieder geval heel erg veel van opgestoken! Het vinden van een betere baas binnen dit soort projecten lijkt me zo goed als onmogelijk.

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Beste André, jij hebt door jouw ideeën (de meeste nog voordat ik deelnam aan het project) misschien wel onbedoeld een ongelofelijke positieve stempel op mijn proefschrift gedrukt. Je hebt ons ook in contact gebracht met Fred Sweep, waardoor het mogelijk was MIF te laten bepalen. Deze Nijmeegse connectie heeft me een hoop werk opgeleverd, maar ook een mooie publicatie en het heeft me dan ook met een prima gevoel achtergelaten! Daarvoor wil ik jullie van harte bedanken!

Beste Frits, helaas mochten we jou van het College van Decanen niet als derde promotor aanstellen. Dat is heel jammer, maar dat neemt niet weg dat ik erg blij ben met alle ondersteuning, commentaren en de prettige samenwerking over de jaren. Ik hoop je nog een keertje te treffen op de golfcourse!

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Beste vriendjes en vriendinnetjes in de AIO-kamer te Mosa Trajectum (Koen, Suzanne, Kaatje, Erik (dan wel geen AIO, maar zeer stabiele factor in het geheel!), de nimmer aanwezige John, soms aanwezige Hans en als laatste de eeuwige? stagiaire Bart). In de buitencategorie, maar toch wel zeker onlosmakelijk verbonden met dit zootje ongeregeld is natuurlijk ook onze bioloog met trekjes Kees Vink. Soms moet je gewoon even je hoofd achter het computerscherm vandaan halen. Tsja wat doe je dan? Dan ga je even naar de AIO-kamer om daar ook even de mensen achter hun computerscherm vandaan te halen (lees te helpen met allerlei complexe wetenschappelijk verantwoorde vraagstukken). De boog kan niet altijd gespannen zijn zullen we maar zeggen, nu deed ik dat misschien wel wat vaak, maar goed... Uiteraard wil ik jullie dan toch maar even bedanken voor alle uren vertier en rust die jullie mij geboden hebben, de rust krijg ik door mijn onrust bij anderen achter te laten, dank voor het ontvangen van mijn onrust. Toch altijd wel prettig om een plekje te hebben waar je zo nu en dan even wat KAAS-verhalen kwijt kunt.

Ook wil ik nog even mijn collega's en werkgever in Tilburg op de UvT bedanken. Ik moet zeggen een uitermate relaxte, inspirerende en prima werksfeer, gewoon lekker de radio op 10 en gaan! Johan, dank dat je me deel hebt laten maken van het opstarten van jouw onderzoekslijn (zo zie ik het) waar we in de toekomst nog heel veel van gaan horen. Ik hoop dat ik jullie een beetje op weg heb kunnen helpen met wat analyses, schrijfwerk, applicaties, "verhalen" en standaardisaties. Yori, thank you for all your advise, incredible knowledge, kind remarks and always funny meetings! Ook alle andere collega's: mijn dank is groot!

De persoon voor wie ik misschien wel het meeste respect heb op deze wereld kan uiteraard niet achterblijven in de rij van bedankjes. Buiten het ontwerpen van de omslag van dit boekwerk ben ik je nog veel meer verschuldigd Frenk! Ik ken je niet uit de tijd van voor de dwarslaesie, maar ik weet wel dat jouw leven daar uiteraard volledig door veranderd is. Dat jij ondanks je volledige verlamming studies afgerond hebt op de kunstacademie, volledig zelfstandig woont en nu ook nog maar eens even een vakantiedorp in Spanje voor lotgenoten zoals jij aan het ontwikkelen bent is natuurlijk op zijn minst gezegd wonderbaarlijk! Dit soort acties helpen mij ook heel erg bij het relativeren moet ik zeggen. Ik heb hier diep diep diep respect voor!!! Ik ben heel blij dat Deesje ons op een helle nette manier bij elkaar gebracht heeft, dus jij Deesje ook 1000x dank!

Mijn paranimfen, Kaatje en Kasper. Lieve Kaatje, ik zeg: het komt na de q en het duurt heel lang (en deze keer heb ik het niet over de: wwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwww wwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwww wwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwwww!). Je bent een schatje, maar goed dat weet je zelf natuurlijk al lang en daarom hoef ik dat dan ook niet nog een keertje op te schrijven...
Sheriff, we hebben sinds dat ik terug kwam uit Auckland in 2000 toch wel een beetje lol gehad hier en vooral ook daar. LB=EMV^2, ohhhjjjaaaaaaa, en wat hier gebeurd is hè, is niet gebeurd!!! Hé alleeh hè!

Verder heb ik een enorme bak aan vrienden die geholpen hebben mij te vormen in de persoon die ik nu ben. Helaas is er niet genoeg ruimte om ze allemaal hoofdelijk te bedanken, maar jullie weten wel over wie ik het heb hier! Deze keer mogen jullie je best aangesproken voelen. Er zijn ook bepaalde groeperingen die ik wil bedanken en dat zijn mijn (oud)-huisgenoten op de GLS19, de Senaat, LB en nog wat van die clubjes, al het tuig van de INKOM, vriendjes en vriendinnetjes van het Auckland/Nieuw Zeeland cluppie en uiteraard het Naaiteef bij Paars Kaarslicht Toepbertje (NT'94)!

Als laatste wil ik uiteraard even de family bedanken. Wij zijn nogal divers, we doen allemaal totaal iets anders, zelfs zo dat er qua werk eigenlijk heel weinig overeenkomsten zijn behalve dat we nogal internationaal zijn ingesteld. Wellicht is dit ook één van de redenen waarom wij nu juist zo'n sterke band hebben en oprecht trots op elkaar zijn! Papa en mama, ik ben er heilig van overtuigd dat jullie ons niet beter hadden kunnen opvoeden dan dat jullie gedaan hebben. Annemieke, Jeroen en ik zijn het product van jullie opvoeding en ik denk dat je je dan niet hoeft te schamen (ben overigens wel gebiased)! Ik ben ook heel blij dat Gemma al heel lang deel uit maakt van het gezin, zonder Gemma is het gezin niet compleet. Daarom mag ik toch wel op mijn knietjes voor jullie allemaal!

Tenslotte is er uiteraard maar 1 manier waarop ik dit kan beëindigen:

ZO NIETS MEER AAN DOEN!!!